ANNUAL REVIEW OF MEDICINE

EDITORIAL COMMITTEE

R. E. COOKE
M. A. HAYES
R. L. LANDAU
A. A. LIEBOW
C. G. LOOSLI
D. A. RYTAND

Responsible for organization of Volume 12
(Editorial Committee, 1959)

R. E. COOKE
G. J. DAMMIN
M. A. HAYES
C. G. LOOSLI
D. A. RYTAND
E. A. STEAD, JR.

ANNUAL REVIEW OF MEDICINE

DAVID A. RYTAND, *Editor*
Stanford University School of Medicine

WILLIAM P. CREGER, *Associate Editor*
Stanford University School of Medicine

MLSU - CENTRAL LIBRARY
12087EX

VOLUME 12

1961

ANNUAL REVIEWS, INC.
PALO ALTO, CALIFORNIA, U.S.A.

ANNUAL REVIEWS, INC.
PALO ALTO, CALIFORNIA, U.S.A.

© 1961 by Annual Reviews, Inc.
All Rights Reserved

Library of Congress Catalog Card Number: A51-1659

FOREIGN AGENCY

Maruzen Company, Limited
6 Tori-Nichome Nihonbashi
Tokyo

L
K1.12
12087

PRINTED AND BOUND IN THE UNITED STATES OF AMERICA BY
GEORGE BANTA COMPANY, INC.

PREFACE

As was the case last year, we find it noteworthy that our invited contributors are covering more restricted areas in much greater detail than they did a decade ago. We regard this as an improvement, and trust that those who are interested in the progress of the medical sciences and arts will agree. We gratefully acknowledge the splendid co-operation of the expert people who have thoughtfully written for this volume, and also thank Miss Beryl V. Daniel, Assistant Editor.

R.E.C. R.L.L.
W.P.C. A.A.L.
M.A.H. C.G.L.
D.A.R.

CONTENTS

Annual Reviews, Inc., and the Editors of its publications assume no responsibility for the statements expressed by the contributors to this *Review*.

INFECTIOUS DISEASE: RESPIRATORY VIRUSES[1]

By R. M. Chanock, M.D., and K. M. Johnson, M.D.
National Institute of Allergy and Infectious Diseases,
National Institutes of Health, Bethesda, Maryland

INTRODUCTION

Recent advances in laboratory methodology have resulted in the recognition of at least 70 new viruses in the past 10 years. Many of these agents are difficult to propagate in tissue culture or other host systems. Problems involved in the isolation of many of these viruses have led to difficulty in delineating their role in human illness. Understanding of virus-disease relationships has also been retarded by the ecologic challenge which certain of these agents present. Thus, some of the newer viruses are ubiquitous while others infect infrequently and unpredictably. It would appear that a large number of viruses are involved in respiratory disease. Many of these agents are capable of producing a spectrum of illness ranging from mild upper respiratory disease to severe lower tract involvement. In addition, each of the respiratory disease syndromes can be produced by a number of distinct agents. This situation poses a complex problem for investigators interested in unraveling the etiology of respiratory disease. Many of the recently recognized viruses have interesting physicochemical and biologic properties which could occupy the full attention of laboratory workers; however, the role of these agents in disease represents the major contemporary problem.

Many current claims for associating newly-recovered agents with illness are based upon inadequate evidence. This review will consider primarily those agents for which reasonable evidence of disease association exists. No attempt will be made to review comprehensively the influenza or adenoviruses since they have been extensively dealt with recently (2, 3, 4).

PROBLEM OF ETIOLOGIC ASSOCIATION

The types of evidence required before a virus can be said to be etiologically related to illness have been described in detail (5). Suffice it to state that the agent in question should be investigated in a controlled epidemiologic setting that includes individuals without illness as well as those with the disease under study. Virus isolation and serologic studies should be performed in the laboratory in such a manner that the origin of the specimens, i.e., whether from controls or individuals with illness, is not known until testing is completed.

One of three basic approaches is commonly employed to establish a virus association with illness. The first of these, administration of the agent under study to human volunteers, often provides the most rapid means of obtaining

[1] The survey of the literature pertaining to this review was concluded in August, 1960.

preliminary evidence of pathogenic activity. Although volunteer studies can establish evidence that an agent is capable of causing respiratory illness, the frequency with which overt illness occurs during natural infection and the relative importance of an agent in the total respiratory disease picture can only be answered by carefully controlled epidemiologic investigations in nature. Many opportunities for false estimation of pathogenicity are inherent in volunteer studies. Underestimation of virulence may result from attenuation of the agent during growth in tissue culture or other experimental host systems. Underestimation has also occurred following administration of an agent by an inappropriate route. Thus, minimal illness resulted from administration of adenoviruses via the nose or throat, whereas definite illness followed instillation of these viruses into the conjunctival sac (6). Overestimation of virulence can also result from the administration of an agent by an unnatural method; for example, attenuated live influenza vaccine has produced lower respiratory disease when entry into the lungs was facilitated by instillation of virus in the form of a fine mist (7).

The cross-sectional approach makes use of individuals selected because of a clinical attribute and studied at only one moment in time. Such studies can yield evidence of an etiologic association if appropriate controls are included, and if repetition of the investigation in other localities and at other times yields essentially similar information.

Controlled longitudinal studies of the same population wherein all persons with or without illness, are investigated at routine intervals can yield information of the highest order regarding the role of an agent in disease. Such an approach permits an accurate temporal association of infection and illness and a definition of the spectrum of disease resulting from naturally acquired infection. In longitudinal studies it is often possible to collect periodic serum samples, thus permitting correlation of infection and its clinical consequences with antibody status at the time of exposure.

Systematic investigation of the natural history of viral respiratory disease is enhanced when all three methods are utilized. A well-supported, versatile laboratory, capable of identification and management of large numbers of distinct viral agents, has become essential to fruitful study of this complex problem.

AGENTS

Para-Influenza Viruses

Para-influenza viruses are members of the myxovirus family (8 to 12). They are medium sized (90 to 200 mμ), ether-sensitive viruses which are able to agglutinate erythrocytes from certain species. These agents possess a mucopolysaccharide-destroying enzyme demonstrable by its action on egg white inhibitor (13). Unlike the inflnenza viruses, tbe para-influenza viruses grow poorly in the embryonated egg, a property whicn probably accounts for previous failure to recognize these agents. The para-influenza viruses,

however, are readily recovered in primary monkey or human kidney culture. Although they produce minimal cytopathic effects during primary passage in culture, their presence can be recognized by the phenomenon of hemadsorption (9, 10, 14).

Members of the para-influenza group share common antigens among themselves and with mumps virus (15, 16, 17), an agent previously shown to be related to Newcastle (NDV) virus (18, 19, 20). In addition, they share with mumps and NDV viruses properties clearly separating them from the influenza viruses. These include the capacity to hemolyze certain types of erythrocytes, a larger size, and the absence of a serological relationship with influenza viruses (15, 21). There is also suggestive evidence that their mode of replication in cells differs from that of influenza viruses. The soluble antigens of para-influenza 1, mumps, and NDV viruses appear to multiply in the cytoplasm, whereas this moiety of the influenza A virus is produced in the nucleus (22). It would appear that the para-influenza viruses, together with mumps and NDV viruses, comprise a subgroup of myxoviruses separable from the influenza viruses. Members of the para-influenza family to be discussed include type 1 (hemadsorption type 2 or HA-2) (9), type 2 (croup-associated or CA) (8, 23), type 3 (hemadsorption type 1 or HA-1) (9), and type 4 (M-25) (10). The Sendai strain of para-influenza 1 virus will be considered separately because its role in human illness is still in doubt.

Role in childhood illness.—In a controlled study extended over a two-year period, types 1 and 3 viruses were recovered 20 times more frequently from children with respiratory disease than from control children without illness, indicating that these agents were associated with disease (24). In these studies the types 1 and 3 viruses were recovered from seven per cent of infants and children seen in an outpatient clinic for predominantly febrile upper respiratory disease. The type 2 virus has been recovered predominantly from children with croup, and evidence for its association with disease has recently been obtained (25). The types 1 and 3 viruses, together with the type 2 virus, appear to play a most significant role in the croup syndrome; during a 2½ year study period 45 per cent of patients with this syndrome had evidence of para-influenza infection (26, 27).

Infection of children with types 1 and 3 viruses was associated with a spectrum of illness ranging from mild afebrile upper respiratory disease to severe pneumonia and croup (28). When tested by serologic techniques, which are more sensitive diagnostically than isolation of virus strains, approximately 20 per cent of children with respiratory disease severe enough to require hospitalization (pneumonia, bronchiolitis, and croup) had evidence of infection with these agents during a two-year study period (27).

Although periods of increased prevalence were observed, para-influenza 1 and 3 viruses were easily detected in the pediatric population during most months over a 2½-year period in Washington, D.C. (27). Continued presence of these agents was in contrast to the epidemic behavior of influenza A2 and B viruses during the same interval.

Para-influenza 4 virus was recovered from a college student with an afebrile upper respiratory illness, and during an outbreak of infection in a Washington, D.C., nursery in 1958 (10). Evidence for its association with respiratory illness is lacking at the present time. Recently, the agent was recovered from an adult with a coldlike illness in New Orleans (29).

The para-influenza viruses have been recovered from children with respiratory illness in many different geographic localities. Type 1 virus was isolated in Denmark (30), England (31), and Japan (32). Type 2 virus was recovered in England (33), Panama (34), and Canada (23), while type 3 virus was recognized in France (35), England (36), Canada (37), and Australia (38).

Natural history of infection and reinfection.—Neutralizing antibody studies with para-influenza 3 indicated that 50 per cent of infants in Washington, D.C. had been infected by the twelfth month and almost 90 per cent of children had detectable antibodies by the fifth year (27). These findings were consistent with the frequent recovery of the agent just described.

Observation of the occupants of a nursery in which three outbreaks of type 3 infection had occurred within a nine-month period yielded information bearing upon the natural history of this agent (27). Approximately 70 per cent of children undergoing their first infection (as measured by virus isolation) developed a febrile response which lasted, on the average, three to four days. One-third of primary infections were associated with lower respiratory tract involvement (pneumonia or bronchitis).

Fifteen to 20 per cent of children infected during one outbreak again shed virus during a subsequent outbreak three to seven months later. Such reinfection was rarely associated with fever or lower respiratory tract involvement. The serologic response to reinfection consisted of two patterns: if the neutralizing antibody titer was low a marked rise was usually observed, but if the antibody level was high prior to reinfection, then it remained stationary. Antigenic variation was not responsible for reinfection since the strains recovered during the first and second infections were indistinguishable. Indeed, strains of para-influenza 3 virus recovered from different parts of the world during the past few years have all been shown to have the same antigenic structure (27).

Adult illness.—Eighty per cent of adults thus far tested have neutralizing antibody for type 1 virus (39), 65 to 90 per cent have hemagglutination-inhibition antibody for type 2 virus (8, 40), and all have neutralizing antibody for type 3 virus (27, 41, 42). These findings are consistent with the frequency with which these viruses have been observed in infants and small children.

The ability of type 1 virus to produce mild upper respiratory illness in adults was shown in a volunteer study (39). Twenty-five of 32 volunteers were infected following the administration of a small quantity of virus (80 tissue culture doses) and 18 developed coldlike illness on the sixth to seventh

day. Thirteen of the 18 men who became ill possessed neutralizing antibody prior to the virus challenge. Prison contacts of the volunteers acquired infection and developed colds, providing evidence for natural spread and pathogenicity. The production of coldlike illness with type 1 virus in volunteers was confirmed in another study (41). Type 3 virus also produced mild upper respiratory disease in volunteers despite the fact that all subjects possessed neutralizing antibody prior to the challenge (41, 43).

The volunteer studies indicate that types 1 and 3 virus are capable of producing upper respiratory disease. The importance of these agents in naturally occurring disease, however, remains to be elucidated. Types 1, 2, and 3 virus have been recovered from adults with mild to moderately severe illness (42, 44 to 47), but controlled epidemiologic information necessary to evaluate the significance of these findings is lacking.

All of the type 3 infections and probably a portion of the types 1 and 2 experience represent reinfection. It is essential, therefore, to determine the proportion of naturally acquired reinfection which results in illness.

Preliminary studies involving primarily type 3 virus indicate that an accurate estimation of reinfection may be difficult to achieve (47, 48). A portion of adults fail to develop a rise in antibody above the level existing at the time of reinfection, thus limiting the sensitivity of serodiagnostic procedures (48). The quantity of virus present in the pharynx during type 3 reinfection is small and often fails to withstand freezing and thawing. The best estimate of reinfection will probably derive from studies in which throat swab specimens are tested at the time of collection rather than after storage in the frozen state.

Sendai virus.—Recovery of Sendai virus was first reported from infants with fatal pneumonitis (49). In this study, isolation attempts were made by inoculating autopsy specimens intranasally in mice. The agent is antigenically closely related to the HA-2 virus (15), and for this reason has been classified with it as para-influenza 1 (12). The validity of the original and many of the subsequent isolations of Sendai virus has been questioned because they were accomplished in mice, which are now known to be extensively infected with the agent in Japan (50), Russia (51), and China (52).

In one study, the agent was recovered in eggs inoculated with material from patients with an influenzalike illness. The work was done in Russia under circumstances that make laboratory contamination unlikely (53). Unequivocal evidence for human pathogenicity awaits confirmation of these results. The circumscribed geographical occurrence of Sendai virus infection (animal and human) is in marked contrast to the world-wide distribution of other human myxoviruses. The possibility that human infection by Sendai virus may be acquired directly from mice or other animals merits investigation.

Respiratory Syncytial Virus

This agent was first recovered from an outbreak of coryza in a colony of chimpanzees (54). Shortly thereafter it was isolated from children with pneumonia and croup in 1956 (55). Subsequently, it has been recovered from children with respiratory disease in 1958, 1959, and 1960 (56).

Syncytium formation is the characteristic cytopathic change in continuous cell cultures (HeLa, Hep-2, KB, etc.) (55). This effect resembles that seen with measles virus, but no antigenic relationship has been demonstrated (55). At the present time, it is not possible to classify the syncytial agent in any established virus group.

The complement-fixing antigen produced in infected tissue cultures is of the soluble type, i.e., it is separable from the virus particle by centrifugation. The virus is 90 to 130 mμ in size, ether-sensitive, and does not replicate in the hen's egg or laboratory mouse (55).

The original studies in children suggested that the agent was associated with lower respiratory tract illness; however, this conclusion was considered tentative in view of the small number of patients observed (57, 58). Subsequent studies made during 1957 to 1960 indicated that infection was associated with approximately 10 per cent of childhood lower respiratory tract illness severe enough to require hospitalization (59). During this period, virus recovery was rare in relation to the frequent serologic evidence of infection.

Recent studies have indicated that the agent is extremely labile and that inoculation of fresh (unfrozen) throat swab fluid into tissue culture is required for optimum isolation efficiency (59a, 60). By employing this technique, it was possible to recover the agent from 32 per cent of 107 infants and children with pneumonia or bronchiolitis in the Washington, D.C., area during a period of high prevalence (March through July, 1960) (60). Serologic studies indicated that the infection rate among these patients was closer to 50 per cent. Children without illness were infected significantly less frequently, thus establishing that the agent was associated with lower respiratory tract illness in the pediatric age group. Additional evidence for this conclusion was provided by an outbreak of infection in an orphanage nursery in which 34 of the 90 children in residence developed pneumonia (61).

Infection with the virus occurs early in life, and all adults thus far tested possess neutralizing antibodies (62). Despite such antibody, 15 of 27 adult volunteers who were given a small quantity (80 to 640 TCD_{50}) of virus developed "common cold like" illness (62). The incubation interval for illness was three to six days.

Respiratory syncytial virus resembles the para-influenza viruses in that first infection in childhood is often associated with severe lower respiratory tract disease, whereas reinfection during adult life is associated with mild afebrile upper respiratory illness. Having established that reinfection in adults can result in mild illness, it is now essential to define its importance

in the over-all picture, i.e., the frequency with which reinfection occurs and the proportion of reinfection that leads to overt disease. Answers to these questions should be forthcoming since preliminary serologic studies indicate that infection is not uncommon in military recruits and civilian adults (63).

Primary Atypical Pneumonia (Eaton Agent)

The etiology of cold agglutinin-positive atypical pneumonia has been the subject of considerable controversy during the past 20 years. Much of this controversy has centered about the Eaton agent which was recovered in embryonated eggs following inoculation of filtered sputum specimens from patients with atypical pneumonia in 1944 (64). Chick embryos infected with the agent did not show any definite changes, but material from such eggs produced pneumonia in cotton rats and hamsters. Early studies were hindered by the fact that lesions were produced in only a portion of, but not all, inoculated hamsters or cotton rats. In addition, these animals were subject to pneumonia resulting from activation of their own latent viruses (65, 66). For these reasons, many workers have been reluctant to consider this agent as a respiratory tract pathogen (67).

Application of the fluorescent antibody technique to the study of Eaton agent resulted in a more quantitative assay system and permitted the demonstration of specific antigen in the bronchial epithelium of the experimentally infected chick embryo (68). Infected bronchial epithelium, however, showed no evidence of cell damage when careful histological studies were performed (68).

A limited number of strains have been recovered, in eggs, from patients with pneumonia and identified by immunofluorescence (68 to 71). Recently, the agent has been shown to propagate without cytopathic effect in tissue cultures of ovine, simian, and human origin (71, 72). In a comparative study, monkey kidney tissue culture was found to be as sensitive as the egg for isolation of naturally occurring strains of Eaton agent (71).

The agent is sensitive to aureomycin and streptomycin (73, 74). Its size has been estimated as 180 to 250 mμ (65, 75). These findings suggest that the agent belongs in the group which is somewhat smaller than the Rickettsiae and somewhat larger than the "true viruses." At present, there is no unanimity of opinion concerning the status of agents in this category. The finding of an eclipse period for the Eaton agent in tissue culture (71) suggests that it may satisfy one current definition of a virus (76).

Although the taxonomic status of the agent remains unsettled, evidence for its role in human respiratory disease has accumulated in the past few years. Thus, in two separate studies, 80 to 95 per cent of patients with cold agglutinin-positive pneumonia developed fluorescent stainable antibody for the agent (69, 77). Positive serologic findings were observed for patients whose illnesses occurred during various years (1947 to 1959) and in various geographic areas, thus minimizing the possibility of a spurious association of

the agent and the syndrome (69). Serologic evidence obtained from a controlled epidemiologic study in a pediatric hospital indicated that the agent was associated with approximately 10 per cent of lower respiratory tract illness over a 16-month period (78).

Additional evidence that Eaton agent causes respiratory illness was obtained during a controlled epidemiologic study of infection in Marine recruits (79). Over a six-month period, 68 per cent of 238 pneumonias and 28 per cent of 144 febrile respiratory illnesses were associated with Eaton infection. Infection, as detected by serologic techniques, occurred significantly less often (6 per cent) among recruits without respiratory illness. Specificity of the antibody response was established by the recovery of 14 strains of the agent from 17 recruits with serologically positive pneumonia. Infection was widely disseminated and approximately 44 per cent of the recruits were infected at some time during the three-month training period. The incidence of Eaton pneumonia during the training period was also high (1.5 per cent). These findings suggested that only one in 30 infections was manifest as a clinically diagnosed pneumonia and that the majority of infections were asymptomatic.

Cold agglutinins were not an efficient index of Eaton infection in Marine recruits. Only 44 per cent of individuals with Eaton-positive pneumonia developed cold agglutinins during their convalescence (79). Cold agglutinin-negative pneumonia associated with Eaton infection has been observed in other epidemiologic settings; in one such instance, the occurrence of infection was confirmed by recovery of the agent (70).

The efficacy of tetracycline drugs in the therapy of atypical pneumooia has been a controversial subject for the past 12 years (67, 80). A beneficial effect has been claimed in studies that included a variable proportion of cold agglutinin-positive cases (81 to 85). Other workers failed to observe a therapeutic effect (86, 87). Arypical pneumonia is a syndrome of multiple etiology and in adults is associated with influenza (2), adenovirus (3), and other as yet unrecognized viruses in addition to Eaton agent. Almost all atypical pneumonia associated with cold agglutinins, however, appears to be associated with Eaton infection. It seems probable that the variable results that have been reported for aureomycin treatment can be ascribed to differences in the proportion of patients with Eaton pneumonia in the various studies.

To resolve the controversy surrounding the efficacy of the tetracyclines, it is essential that comparison of treated and untreated groups be confined to patients with etiologically defioed illness. Such a study was recently carried out to test the effect of demethylchlortetracycline (Declomycin) on Eaton pneumonia (88). Serologic tests were performed to rule out infection with other viruses capable of causing pneumonia (influenza, para-influenza, adenovirus, and respiratory syocytial). The fluorescent antibody technique was employed for specific sero-diagnosis of Eaton infection rather than the less sensitive cold agglutinin test. Marine recruits with pneumonia at a training center with a high incidence of Eaton infection were treated with

0.9 gm. of demethychlortetracycline per day for six days or given a placebo. Patients were placed in the treatment or placebo group as determined by a process of complete randomization. The study was performed double blind in that the clinical observers were not aware of the treatment or diagnostic status of the patients under observation. Sixty-seven Eaton-positive patients were treated and 63 served as controls. Treatment significantly reduced the duration of fever, cough, rales, and malaise. In addition, treatment markedly accelerated the clearing of pulmonary infiltration as visualized by x-ray. Fever did not return when the treatment was stopped after six days, whereas 40 per cent of the untreated cases were still febrile at that time. This finding, coupled with the effect of therapy upon the lung lesions, suggests a direct action of drug upon the disease process rather than an antipyretic effect as suggested by certain workers (89).

Enteroviruses and Enteroviruslike Agents

Certain viruses found in the intestinal tract of man and possessing common biologic properties have recently been grouped together as enteroviruses (90). The family of enteroviruses now includes the poliomyelitis, Coxsackie, and ECHO viruses. Epidemiologic and clinical studies with these agents have emphasized paralytic illness, myocarditis, pleurodynia, and the syndrome of aseptic meningitis. Some members of the enterovirus family (especially certain of the Coxsackie A and ECHO viruses) also commonly cause a febrile illness in which pharyngitis is a prominent symptom (91, 92). The best-studied example of this relationship is the syndrome of herpangina caused by certain Coxsackie A viruses in which vesicular lesions of the oropharynx are recognized in a large proportion of cases (91). Until the present time, however, no convincing evidence has appeared that enteroviruses are associated with lower respiratory tract disease such as pneumonia, bronchitis, etc.

Several viruses recently recovered from persons with mild respiratory disease appear to possess many of the properties of the family of enteroviruses although, in several instances, recovery of these agents has not been achieved from the intestinal tract. They are small viruses, 20 to 40 mμ, ether-resistant, and produce an irregularly ovoid, refractile cytopathic effect in susceptible tissue cultures. Their association with respiratory disease rests, in most instances, upon limited data, and they have often proven difficult to study in the laboratory. Epidemiologic investigations have been hampered by the necessity of differentiating them from the already large number of recognized enteroviruses. One of them, however, has recently been recognized as a true enterovirus, 2060 virus having been designated ECHO 28 (93).

ECHO 28 virus.—The 2060 and JH strains of this virus appear to be nearly identical with regard to antigenic and biologic properties (94, 95). The two strains, however, can be distinguished with certain human serums or with

serum from rabbits receiving a limited course of immunization (94, 95). The ECHO 28 virus is extremely difficult to isolate and blind passage in monkey-kidney cultures is often necessary (96, 97). Serial passage in monkey-kidney cultures is required in order to produce reference virus pools for identification, and the attendant opportunity for the introduction of simian agents into these pools poses still another problem.

In studies from one laboratory, infection with ECHO 28 virus was detected in 12 to 30 per cent of clinically mild respiratory illness in older children and adults (95, 97). In cases where virus isolation was achieved, titration of throat washings indicated that little more than one tissue culture dose was present. The studies, moreover, suggested a correlation between levels of neutralizing antibody and occurrence of clinical illness following infection by JH and 2060 viruses. Serologic surveys indicated that children under five years of age were rarely infected with these agents, but that more than two-thirds of all adults possessed neutralizing antibody. Such results would suggest that a portion of "coldlike" illness in adults may be attributed to viruses that produce primary infection after childhood.

In another laboratory, 2060 virus was recovered in several instances from young military recruits (96). Mild upper respiratory illness was often observed at the time of virus isolation, but insufficient data were available to warrant association of the virus with illness.

The results of volunteer studies with ECHO 28 virus are somewhat difficult to interpret. The inoculum given to volunteers represented fifth to fourteenth tissue culture passage material and the possibility exists that attenuation occurred during these passages. In one study, 100 to 1000 tissue culture doses of twelfth passage JH strain infected 2 of 16 volunteers without producing illness (98). Respiratory secretions from one of the infected individuals was administered to 11 volunteers, 5 of whom became infected and 2 of whom developed colds. Respiratory secretions from one of the latter individuals infected 3 of 9 volunteers, one of whom developed a cold. Subsequent passage of secretions that contained 50 tissue culture doses of ECHO 28 virus failed to infect or produce illness in 10 volunteers. In the second and third passage of secretions, two colds were observed which were not associated with ECHO 28 infection.

More definite evidence that ECHO 28 virus may cause coldlike illness was obtained in a study of 159 volunteers who were given either JH or 2060 strains of virus (99). The results with either strain alone did not establish that these agents could produce disease, but when the data for both viruses were combined, a statistically significant association with coldlike illness was observed. The incubation period was short, averaging less than 24 hr., and virus could be recovered from volunteers up to six days following administration. Approximately one-fifth of the volunteers developed a rise in neutralizing antibody; however, no correlation was observed between pre-existing antibody levels and subsequent infection or illness. Nevertheless,

when volunteers were rechallenged with the same or heterologous strain of ECHO 28, significant protection was observed, confirming the close relationship of these agents.

Epidemiologic studies with ECHO 28 virus have been bampered somewhat by the difficulty encountered in performing reproducible tests for neutralizing antibodies. At present, comparative surveys of populations for presence, absence, and levels of such antibodies appear to be of doubtful reliability. Use of second generation tissue cultures has been suggested as an improved technique (94), but much further laboratory study is urgently required.

HGP and FEB viruses.—Nasal secretions, which produced colds in volunteers, served as the source of two new viruses recovered by workers in England (100, 101, 102). These agents were cultivated in human kidney tissue culture, at first without obvious cytopathic effect. Presence of the viruses in tissue culture was demonstrated by their interference with the multiplication of certain other viruses and by the production of colds in volunteer subjects. Subsequently, cytopathic changes were regularly observed when certain changes in tissue culture technique were introduced. These were (*a*) the use of a low concentration of sodium bicarbonate in the tissue culture medium, resulting in a pH of about 6.8; (*b*) rotation of the cultures on a roller drum; and (*c*) incubation at a temperature of 33° C. rather than the usual 35° to 37° C. Both agents were ether-resistant, temperature-stable, and produced characteristic enteroviruslike cytopathic effects in culture. The HGP strain was adapted to monkey kidney culture, but the antigenically distinct FEB virus multiplied only in cells of human origin.

Although no definitive epidemiologic data are available concerning human infection with these viruses, 27 biologically similar strains have been recovered in England from 141 persons with mild respiratory illness (103). These viruses were isolated only when conditions found to be optimal for the recovery of FEB and HGP agents were employed. Serologic identification of the new strains has not been completed, but a majority of the isolates appear to be distinct from FEB and HGP viruses. Detection in naturally infected individuals (under new tissue culture conditions) provides the first evidence that these fastidious viruses may be associated with respiratory disease in nature.

Coe virus.—This agent is biologically similar to other enteroviruses in size, ether-resistance, and cytopathic effect in tissue culture (104, 105). Tissue cultures of human origin, however, are required for its recovery and maintenance in the laboratory (104, 105). There is evidence that the virus on occasion may produce minimal paralysis in suckling mice (104), which suggests a possible relationship to the Coxsackie viruses of the enterovirus family.

Strains of Coe virus have been recovered from military recruits in the United States (104), England (105), and Holland (106). In the Dutch study, virus was recovered from the stools as well as the throats of infected indi-

viduals, thus fulfilling another criterion for the inclusion of this agent within the enterovirus family. Associated respiratory illness was generally mild and of an undifferentiated nature although low grade fever was present in several instances.

Serologic studies in England indicate a very low incidence of infection in young children (105). Antibody was not uncommon in adults and, in the 20- to 40-year age group, males had a significantly greater frequency of neutralizing antibody than females. It has been suggested, therefore, that Coe virus may show a predilection for the military recruit and follow an epidemiologic pattern similar to that characteristic of adenovirus types 4 and 7.

A volunteer study in which young adults were given Coe virus provided evidence that the agent was capable of causing coldlike illness (107). All 11 volunteers developed colds and 10 shed virus although two of the group had a high titer of neutralizing antibody at the time the agent was given. Six of the volunteers who were without neutralizing antibody prior to infection failed to develop such antibody during convalescence.

Pett virus.—This virus resembles the Coe virus in many properties but is antigenically distinct from it (108). The Pett virus requires tissue cultures of human origin and produces minimal paralysis in suckling mice on occasion (108a). The agent was recovered from the stools, but not the throats of eight children with mild respiratory illness. No data associating it with respiratory disease are available.

Reoviruses

The first member of the group was initially designated ECHO 10 virus because of its original recovery in monkey kidney tissue culture from stools of healthy children (109). These agents were removed from the ECHO virus group when it was found that they differed from other ECHO viruses in certain biologic and physical properties (110). The latter include a larger size of about 75 mμ and distinctive cytopathogenic properties in tissue culture.

Three distinct serotypes have been recognized (110, 111). Virus isolations have been made from several animal species including chimpanzees (type 2) (112), monkeys (type 1 and 2) (113, 114), and cattle (types 1, 2, and 3) (115, 116), suggesting that reoviruses have a broad host range, crossing many species boundaries. Naturally infected laboratory chimpanzees had mild upper respiratory illness and this clinical picture was reproduced when the type 2 virus was given to chimpanzees (112).

In humans, reoviruses have been recovered from both the respiratory and enteric tracts, but no clear evidence of human pathogenicity has yet been reported. Careful analysis of an outbreak of type 1 infection in an orphanage yielded data of borderline statistical significance associating such

infection with mild upper respiratory illness accompanied by low grade fever (117).

ILLNESS IN VARIOUS AGE GROUPS AND DIFFERENT POPULATIONS

No laboratory has been able to approach the problem in a completely comprehensive manner, employing all the techniques required for the recovery and serologic detection of the newer viruses as well as influenza, adenovirus, psittacosis, and the Rickettsia of Q fever. What follows, therefore, represents an attempted synthesis of information from various studies, many of which are not comparable in the manner in which patients (and controls) were selected for investigation. For purposes of appraisal, viral respiratory disease may be divided conveniently into that observed in pediatric and adult populations.

Infancy and Childhood

Except possibly for military recruits, the proportion of illness associated with known viral agents is highest in this age group. In one study, which extended over a 2½-year period in Washington, D.C. (24, 27, 28), 45 per cent of patients with illness severe enough to require hospitalization (croup, pneumonia, bronchiolitis, or bronchitis) had serologic evidence of infection with para-influenza (20 per cent), influenza (8 per cent), adenovirus (7 per cent), respiratory syncytial (10 per cent), or Eaton agent (10 per cent). These agents (except for Eaton agent) were recovered from 15 to 20 per cent of outpatients with mild or moderately severe illness. Viral diagnosis was most successful in the croup syndrome, 55 per cent of cases being associated with myxovirus infection—para-influenza (45 per cent) and influenza (10 per cent) (26, 28, 118).

It would be hazardous to construct a comprehensive picture from the results of a study limited to one locality. Similar estimates, however, for the contribution of adenoviruses and influenza are available from studies in the United States and England (119, 120).

In many instances, illness in this age group represents the first experience with the infecting agent, explaining in part why the clinical consequences are generally more severe than during adult life. Greater severity of illness heightens the need for effective immunoprophylactic measures. If the Washington, D.C., data are representative of other localities, then effective vaccines, inactivated or live-attenuated, for para-influenza, influenza, adenovirus, respiratory syncytial, and Eaton agent should produce a dramatic decrease in pediatric respiratory disease.

Military Recruit

This population comprises a special group in which adenovirus infection is extremely common. Types 4, 7, and 14 adenovirus have been associated

with 15 to 70 per cent of all febrile respiratory disease, including pneumonia, in recruits (3, 121). Insight into the reasons for this epidemlologic pattern is lacking.

There is evidence to indicate that not all recruit populations experience a high incidence of adenovirus infection. During a 12-month period, only 6 per cent of patients with pneumonia at a southern recruit training center gave evidence of adenovirus infection, whereas 54 per cent of such individuals were infected with Eaton agent (79, 89). Present data indicate that the latter agent is responsible for almost all cold agglutinin-positive pneumonia; moreover, a considerable proportion of Eaton pneumonia occurs without the development of such antibody. The contribution of Eaton agent to illness at other recruit centers and at other times remains to be elucidated. The cause of most mild afebrile illness in recruits is not known. Coe virus and other enteroviruslike agents may be important in such disease.

Civilian Adult

Unlike the military recruit, the civilian adult is infrequently infected by adenoviruses (122). Influenza A or B viruses may cause epidemic disease at yearly or biyearly intervals, but most acute undifferentiated afebrile or febrile respiratory disease remains unexplained. These illnesses constitute one of the major problems confronting the respiratory virologist.

Studies in which natural secretions were administered to volunteers, who were subsequently challenged with the same or heterologous secretion, suggest that common coldlike illness represents a syndrome of multiple etiology (123). In the past three years, six distinct cultivatable viruses have been shown capable of inducing colds in adult volunteers, offering confirmation of the varied etiology of this illness. Not only are the six agents distinct but they belong to three different families of viruses. Two are myxoviruses (para-influenza 1 and 3) (39, 41, 43), three are enterovirus or enteroviruslike (ECHO 28, FEB, and Coe) (93, 99, 100, 101, 107), while the remaining virus (respiratory syncytial) (62) has no known relatives. The importance of these agents in naturally occurring illness remains to be determined. Recognition of these six agents provides a preliminary insight into the problem and undoubtedly, with the refinement of present techniques and the development of new methods, other viruses associated with mild respiratory disease will be recognized.

The ease with which para-influenza 3 virus was observed to reinfect small children suggests that a similar situation may be responsible for an undetermined fraction of mild adult illness. In five of the studies with cultivatable viruses in which serologic tests were reported, infection and illness occurred in volunteers who possessed neutralizing antibody prior to challenge. The problems involved in serologic recognition of reinfection and the recovery of virus from such individuals constitute major barriers to rapid expansion of knowledge in this area.

At the present time, the directions of future research in respiratory viral disease seem clear. Knowledge of such disease in pediatric populations is extensive. Major efforts are indicated to develop vaccines capable of ameliorating the extensive morbidity and the mortality associated with primary infection by a handful of known viruses. Respiratory viral disease in adults is less well understood. Evaluation of the relative role of the large number of newly discovered potential respiratory viruses, especially enteroviruses and enteroviruslike viruses, and of reinfection by viruses already shown to be major pathogens in children, is urgently needed before any serious consideration of immunoprophylaxis can be entertained.

LITERATURE CITED

1. Huebner, R. J., *Postgrad. Med.*, 23, 356 (1958)
2. Proceedings of International Conference on Asian Influenza, *Am. Rev. Respiratory Diseases* (In press)
3. Huebner, R. J., Rowe, W. P., and Chanock, R. M., *Ann. Rev. Microbiol.*, 12, 49 (1958)
4. Tyrrell, D. A. J., *Practitioner*, 183, 567 (1959)
5. Huebner, R. J., *Ann. N. Y. Acad. Sci.*, 67, 430 (1957)
6. Bell, J. A., Ward, T. G., Huebner, R. J., Rowe, W. P., Suskind, R. G., and Paffenbarger, R. S., Jr., *Am. J. Health*, 46, 1130 (1956)
7. Smorodintsev, A. A., *Proc. Intern. Meeting Biol. Standardization, 3rd Meet.*, 463 (1957)
8. Chanock, R. M., *J. Exptl. Med.*, 104, 555 (1956)
9. Chanock, R. M., Parrott, R. H., Cook, M. K., Andrews, B. E., Bell, J. A., Reichelderfer, T., Kapikian, A. Z., Mastrota, F. M., and Huebner, R. J., *New Engl. J. Med.*, 258, 207 (1958)
10. Johnson, K. M., Chanock, R. M., Cook, M. K., and Huebner, R. J., *Am. J. Hyg.*, 71, 81 (1960)
11. Andrewes, C. H., Bang, F. B., and Burnet, F. M., *Virology*, 1, 176 (1955)
12. Andrewes, C. H., Bang, F. B., Chanock, R. M., Zhdanov, V. M., *Virology*, 8, 129 (1959)
13. Andrews, B. E., Cook, M. K., and Chanock, R. M. (To be published)
14. Vogel, J., and Shelokov, A., *Science*, 126, 358 (1957)
15. Cook, M. K., Andrews, B. E., Fox, H. H., Turner, H. C., James, W. D., and Chanock, R. M., *Am J. Hyg.*, 69, 250 (1959)
16. DeMeio, J. L., and Walker, D. L., *J. Immunol.*, 78, 465 (1957)
17. Gardner, P. S., *Brit. Med. J.*, I, 1143 (1957)
18. Kilham, L., Jungherr, E., and Luginbuhl, R. E., *J. Immunol.*, 63, 37 (1949)
19. Jordan, W. S., Jr., and Feller, A. E., *J. Lab. Clin. Med.*, 36, 369 (1950)
20. Bang, F. B., and Foard, M., *J. Immunol.*, 76, 348 (1956)
21. Horne, R. W., Waterson, A. P., Wildy, P., and Farnham, A. E., *Virology*, 11, 79 (1960)
22. Traver, M. I., Northrop, R. L., and Walker, D. L., *Proc. Soc. Exptl. Biol. Med.*, 104, 268 (1960)
23. Beale, A. J., McLeod, D. L., Stackiw, W., and Rhodes, A. J., *Brit. Med. J.*, I, 302 (1958)
24. Chanock, R. M., Vargosko, A., Luckey, A., Cook, M. K., Kapikian, A. Z., Reichelderfer, T., and Parrott, R. H., *J. Am. Med. Assoc.*, 169, 548 (1959)
25. Kim, H. W., Vargosko, A. J., Chanock, R. M., and Parrott, R. J. (To be published)
26. Vargosko, A. J., Chanock, R. M. Huebner, R. J., Luckey, A. H., Kim, H. W., Cumming, C., and Parrott, R. H., *New Engl. J. Med*, 261, 1 (1959)
27. Chanock, R. M., Bell, J. A., and Parrott, R. H., *Perspectives in Virology II* (Monograph), (In press)
28. Parrott, R. H., Vargosko, A., Luckey, A., Kim, H. W., Cumming, C., and Chanock, R. M., *New Engl. J. Med.*, 260, 731 (1959)
29. Mogabgab, W. J. (Personal communication)
30. Birkum Petersen, K., and von Magnus, P., *Danish Med. Bull.*, 5, 157 (1958)
31. Tyrrell, D. A. J., Bynoe, M. L., Birkum Petersen, K., Sutton, R.N.P., and Pereira, M. S., *Brit. Med. J.*, II, 909 (1959)
32. Fukumi, H., Nishikawa, F., Sugiyama, T., Yamaguchi, Y., Nanba, J., Matsuura, T., and Oikawa, R., *Japan. J. Med. Sci. & Biol.*, 12, 307 (1959)
33. Gardner, P. S., Stanfield, J. P., Wright, A. E., Court, S. D. M., and Green, C. A., *Brit. Med. J.*, I, 1077 (1960)
34. Craighead, J., and Shelokov, A. (Personal communication)
35. Chany, C., Daniel, P., Robbe, F., Vialatte, J., Lepine, P., and Lelong, M., *Ann. inst. Pasteur*, 95, 72 (1958)
36. Sulton, R. N. P., Clarke, S. K. R., and Tyrrell, D. A. J., *Lancet*, I, 395 (1959)
37. Labzoffsky, N. A., Cooper, M. R., Morrissey, L. P., and Lesiak, J., *Can. Med. Assoc. J.*, 81, 110 (1959)
38. Forbes, J. A., and Ferris, A. (Personal communication)
39. Reichelderfer, T. E., Chanock, R. M., Craighead, J. E., Huebner, R. J.,

Turner, H. C., and James, W., *Science*, 128, 779 (1958)
40. Clarke, S. K. R., and Saynor, R., *Arch. ges. Virusforsch.*, 9, 288 (1959)
41. Tyrrell, D. A. J., Bynoe, M. L., Birkum Petersen, K., Sutton, R. N. P., and Pereira, M. S., *Brit. Med. J.*, II, 909 (1959)
42. McKinney, R. W., England, B. L., and Froede, S., *Am. J. Hyg.*, 70, 280 (1959)
43. Kapikian, A. Z., Chanock, R. M., Ward, T. G., Huebner, R. J., and Bell, J. A. (To be published)
44. Meenan, P. N., Clarke, M., Tyrrell, D. A. J., *Lancet*, II, 98 (1959)
45. Dick, E. C., Mogabgab, W. J., and Holmes, B., *Clin. Research*, 8, 79 (1960)
46. Mogabgab, W. J., Dick, E. C., and Holmes, B., *Clin. Research*, 8, 66 (1960)
47. Bloom, H. H., Johnson, K. M., Jacobsen, R., and Chanock, R. M. (To be published)
48. Johnson, K. M., Bloom, H. H., and Chanock, R. M. (To be published)
49. Kuroya, M., and Ishida, N., *Yokohama Med. Bull.*, 4, 217 (1953)
50. Fukumi, H., Nishikawa, F., and Kitayama, T., *Japan J. Med. Sci. Biol.*, 7, 345 (1954)
51. Zhdanov, V. M. (Personal communication)
52. Chin-Hsien, T., *Chinese Med. J.*, 80, 331 (1960)
53. Gerngross, O. G., *Problems Virol. (U.S.S.R.)*, 2, 71 (1957)
54. Morris, J. A., Blount, R. E., Jr., and Savage, R. E., *Proc. Soc. Exptl. Biol. Med.*, 92, 544 (1956)
55. Chanock, R. M., Roizman, B., and Myers, R., *Am. J. Hyg.*, 66, 281 (1957)
56. Chanock, R. M., Johnson, K. M., and Huebner, R. J. (To be published)
57. Chanock, R. M., and Finberg, L., *Am. J. Hyg.*, 66, 291 (1957)
58. Chanock, R. M., Parrott, R. H., Cook, M. K., and Bell, J. A., *Viral Infections of Infancy and Childhood*, 189 (Hoeber & Harper, Inc., New York, N. Y., 244 pp., 1960)
59. Parrott, R. J., Kim, H. W., Vargosko, A. J., Turner, H. C., Cumming, C., and Chanock, R. M. (To be published)
59a. Beem, M. O., and Hamre, D. (Personal communication)
60. Chanock, R. M., Kim, H. W., Vargosko, A. J., DeLeva, A., and Parrott, R. J. (To be published)
61. Kapikian, A. Z., Johnson, K. M., Bell, J. A., Wong, D., and Chanock, R. M. (To be published)
62. Johnson, K. M., Chanock, R. M., Knight, V., Kravetz, H., and Rifkind, D. (To be published)
63. Kapikian, A. Z., Johnson, K. M., and Chanock, R. M. (To be published)
64. Eaton, M. D., Meiklejohn, G., and Van Herick, W., *J. Exptl. Med.*, 79, 649 (1944)
65. Eaton, M. D., Meiklejohn, G., Van Herick, W., and Corey, M., *J. Exptl. Med.*, 82, 317 (1945)
66. Eaton, M. D., *Handbuch Virusforsch.*, 2, 87 (1950)
67. Dingle, J. H., and Jordan, W. S., *Viral and Rickettsial Infections of Man*, 600 (J. B. Lippincott Co., Philadelphia, Pa., 967 pp., 1959)
68. Liu, C., *J. Exptl. Med.*, 106, 455 (1957)
69. Cook, M. K., Chanock, R. M., Fox, H. H., Huebner, R. J., Buescher, E. L., and Johnson, R. T., *Brit. Med. J.*, I, 905 (1960)
70. Johnson, R. T., Cook, M. K., Chanock, R. M., Buescher, E. L., *New Engl. J. Med.*, 262, 817 (1960)
71. Chanock, R. M., Fox, H. H., James, W. D., Bloom, H. H., and Mufson, M. A., *Proc. Soc. Exptl. Biol. Med.* (In press)
72. Gordon, F. B., Quan, A. L., Cook, M. K., Chanock, R. M., and Fox, H. H., *Proc. Soc. Exptl. Biol. Med.* (In press)
73. Eaton, M. D., *Proc. Soc. Exptl. Biol. Med.*, 73, 24 (1950)
74. Eaton, M. D., and Liu, C., *J. Bacteriol.*, 74, 784 (1957)
75. Donald, H. B., and Liu, C., *Virology*, 9, 20 (1959)
76. Burnet, F. M., *Principles of Animal Virology* (Academic Press, Inc., New York, N. Y., 490 pp., 1960)
77. Liu, C., Eaton, M. D., and Heyl, J. T., *J. Exptl. Med.*, 109, 545 (1959)
78. Chanock, R. M., Cook, M. K., Fox, H. H., Parrott, R. H., and Huebner, R. J., *New Engl. J. Med.*, 262, 648 (1960)
79. Chanock, R. M., Mufson, M. A., Bloom, H. H., James, W. D., Fox, H. H., and Kingston, J. R. (In press)
80. Finland, M., *New Engl. J. Med.*, 247, 317 (1952)
81. Kneeland, Y., Jr., Rose, H. M., and Gibson, C. D., *Am. J. Med.*, 6, 41 (1949)
82. Shoenbach, E. B., and Bryer, M. S.,

J. Am. Med. Assoc., 139, 275 (1949)

83. Finland, M., Collins, H. S., and Wells, E. B., *New Engl. J. Med.*, 240, 241 (1949)
84. Meiklejohn, G., and Shragg, R. I., *J. Am. Med. Assoc.*, 140, 391 (1949)
85. Meiklejohn, G., Thalman, W. G., Waligora, D. J., Kempe, C. H., and Lennette, E. H., *J. Am. Med. Assoc.*, 154, 1 (1954)
86. Harvey, J. C., Mirick, G. S., and Shaub, I. G., *J. Clin. Invest.*, 28, 987 (1949)
87. Peck, G. A., and Berry, J. W., *Antibiotics & Chemotherapy*, 1, 291 (1951)
88. Kingston, J. R., Hellman, L. P., Mufson, M. A., Boyers, J., Manko, M. A., and Chanock, R. M. (To be *published*)
89. Tillotson, I. G., and Ginsberg, H. S., *Proc. Am. Fed. Clin. Research*, No. 117, (1952)
90. Committee on the Enteroviruses, *Am. J. Public Health*, 47, 1556 (1957)
91. Huebner, R. J., Cole, R. M., Beeman, E. A., Bell, J. A., and Peers, J. H., *J. Am. Med. Assoc.*, 145, 628 (1951)
92. Melnick, J. L., and Sabin, A. B., *Viral and Rickettsial Infections of Man*, 547 (J. B. Lippincott Co., Philadelphia, Pa., 967 *pp.*, 1959)
93. Committee on the Enteroviruses, *Natl. Foundation for Infantile Paralysis* (To be published)
94. Pelon, W., and Mogabgab, W. J., *Proc. Soc. Exptl. Biol. Med.*, 102, 392 (1959)
95. Price, W. H., Emerson, H., Ibler, I., Lachaine, R., and Terrell, A., *Am. J. Hyg.*, 69, 224 (1959)
96. Pelon, W., Mogabgab, W. J., Phillips, I. A., and Pierce, W. E., *Proc. Soc. Exptl. Biol. Med.*, 94, 262 (1957)
97. Price, W. H., *Proc. Natl. Acad. Sci.*, 42, 892 (1956)
98. Tyrrell, D. A. J., and Bynoe, M. L., *Lancet*, II, 931 (1958)
99. Jackson, G. G., Dowling, H. F., and Mogabgab, W. J., *J. Lab. Clin. Med.*, 55, 331 (1960)
100. Tyrrell, D. A. J., Hitchcock, G., Bynoe, M. L., Pereira, H. G., and Andrewes, C. H., *Lancet*, I, 235 (1960)
101. Hitchcock, G., and Tyrrell, D. A. J., *Lancet*, I, 237 (1960)
102. Tyrrell, D. A. J., and Parsons, R., *Lancet*, I, 239 (1960)
103. Tyrrell, D. A. J. (Personal communication)
104. Lennette, E. H., Fox, V. L., Schmidt, N. J., and Culver, J. O., *Am. J. Hyg.*, 68, 272 (1958)
105. Pereira, M. S., and Pereira, H. G., *Lancet*, II, 539 (1959)
106. Van der Veen, J., Mej, K. G., Oei en Prins, A., *Overg. Ned. Tijdschr. Geneesk.*, 104, 617 (1960)
107. Parsons, R., Bynoe, M. L., Pereira, M. S., and Tyrrell, D. A. J., *Brit. Med. J.*, I, 1776 (1960)
108. Kasel, J. A., Cramblett, H. G., and Utz, J. P., *Proc. Soc. Exptl. Biol. Med.*, 99, 703 (958)

108a. Kasel, J. A. (Personal communica*tion*)

109. Ramos-Alvarez, M., and Sabin, A. B., *Proc. Soc. Exptl. Biol. Med.*, 87, 655 (1954)
110. Sabin, A. B., *Science*, 130, 1387 (1959)
111. Rosen, L., *Am. J. Hyg.*, 71, 242 (1960)
112. Sabin, A. B., *Ann. N. Y. Acad. Sci.*, 66, 226 (1956)
113. Malherbe, H., and Harwin, R., *Brit. J. Exptl. Pathol.*, 38, 539 (1957)
114. Hull, R. N., Minner, J. R., and Mascoli, C. C., *Am. J. Hyg.*, 68, 31 (1958)
115. Rosen, L., and Abinanti, F. R., *Am. J. Hyg.*, 71, 250 (1960)
116. Rosen, L., and Abinanti, F. R. (To be *published*)
117. Rosen, L., Hovis, J. F., Mastrota, F. M., Bell, J. A., and Huebner, R. J., *Am. J. Hyg.*, 71, 266 (1960)
118. Parrott, R. H., Chanock, R. M., Vargosko, A. J., and Kim, H. W. (To be published)
119. Grayston, J. T., Loosli, C. G., Smith, M., McCarthy, M. A., and Johnston, P. B., *J. Infectious Diseases*, 103, 75 (1958)
120. Holland, W. W., Tanner, E. I., Pereira, M. S., and Taylor, C. E. D., *Brit. Med. J.*, I, 1917 (1960)
121. Hilleman, M. R., Gauld, R. L., Butler, R. L., Stallones, R. A., Hedberg, C. L., Warfield, M. S., and Anderson, S. A., *Am. J. Hyg.*, 66, 29 (1957)
122. Jordan, W. S., *Arch. Internal Med.*, 101, 54 (1958)
123. Jackson, G. G., and Dowling, H. F., *J. Clin. Invest.*, 38, 762 (1959)

PHYSIOLOGY OF THE SURGICALLY ALTERED STOMACH[1]

By Claude E. Welch, M.D., and Daniel S. Ellis, M.D.

Departments of Surgery and Medicine, Harvard Medical School, and Massachusetts General Hospital, Boston, Massachusetts

Nearly all operations on the stomach are either designed to produce, or are necessarily followed by certain alterations in physiology. While it would be possible to discuss the sequelae of each standard procedure, it seems more logical, in this brief review, to identify primarily the important physiological changes that have been observed postoperatively, and to note in context the relation of various operations to each of these specific alterations.

Since, in many instances, objective data are difficult to acquire in man, and often are over-shadowed by subjective impressions, frequent references will be made to animal experiments, particularly to those in dogs, wherein quantitative data may be obtained by means that are impossible in the study of human beings. The reader should bear in mind that conclusions gained from the behavior of animals after gastric surgery must be transferred with some reservations to man. It also should be recognized that despite the formidable number of changes which are produced by gastric surgery, the capacity of the body to adjust satisfactorily is so great that in the great majority of instances any deleterious postoperative abnormalities are unimportant when compared to the subjective relief obtained by the patient.

Operations on the stomach may produce these physiological alterations:

A. Modification of gastric secretions, including acid, pepsin, mucus and hormones. B. Defects of absorption. These defects involve carbohydrates, fats, proteins, vitamins, minerals, weight changes, and growth. C. Anemias. D. Changes secondary to stasis or reflux. E. Secondary effects on other organs. F. Recurrent ulceration. G. Death.

MODIFICATIONS OF GASTRIC SECRETIONS

The important secretions of the stomach include hydrochloric acid, pepsin, and mucin. The antrum also produces one or more hormones. Hydrochloric acid is secreted nearly entirely by parietal cells in the corpus, although there are a few in the fundus and antrum. Pepsin is secreted by chief cells of the corpus. Mucin is secreted in all parts of the stomach, but particularly in the fundus. It is generally agreed that the antral mucosa produces gastrin while, in all probability, a second hormone that depresses acid secretion is also produced in the same area (1, 2, 3).

Acid Secretion

Before postoperative findings are considered, it may be pertinent to re-

[1] The survey of the literature pertaining to this review was concluded in July, 1960.

emphasize the defects of the various methods of estimating acid secretion in man; they include the inability to obtain a quantitative return, variable dilution with saliva and with duodenal or intestinal contents, and possible erroneous readings incidental to excitement caused by the Levin tube. For these reasons, individual determinations of gastric secretory activity may be misleading. However, the same method applied to groups of patients with different operations can be expected to provide data that are roughly comparable.

In the dog, much more accurate analyses can be made by the use of the Heidenhain pouch, in which a denervated pouch of the gastric fundus is isolated and turned into a fistula from which quantitative removal of gastric secretion is possible; by the Pavlov pouch which is a partially denervated pouch; by Dragstedt's total gastric pouch; or by Gregory's totally denervated fundal pouch (4 to 7).

Measurements of acid secretion include the total amount of HCl in m.eq. pH. Basal secretory levels in older patients, as quantitated by Dragstedt *et al.* in their first studies were as follows: volume of basal secretion (12-hr. night secretion)—normal, 551 cc; duodenal ulcer, 1085 cc; gastric ulcer, 773; total amount of HCl (in m.eq.) in fasting specimens,—normal 18; duodenal ulcer, 60; gastric ulcer, 17.8 (8). Farmer *et al.* found that the total acid output per hour measured in m.eq. for the duodenal ulcer patient was 714 per cent of that of normal subjects (9).

Later studies have confirmed the fact that hypersecretion of acid occurs only with duodenal ulcer; secretory levels for patients with gastric ulcer tend to be within limits of normal, and achlorhydria is found in 90 per cent of patients with cancer (1, 2). Hence, the effects of gastric operations on HCl output must be studied in patients with duodenal ulcer. The production of a complete or essentially complete achlorhydria as a criterion by which the success of an operative procedure for duodenal ulcer can be measured is second only to long-term observation of the patient. Postoperative studies are now available after many of the standard operations.

Total gastrectomy.—Total gastrectomy obviously completely eliminates gastric secretion. It is the only procedure that has been found that will control the enormous stimulus to gastric secretion provided by the endocrine tumors seen in the Zollinger-Ellison syndrome (10).

Subtotal gastrectomy.—This term is used frequently but is inexact as it may refer to anything between a 50 per cent and 85 per cent resection. The higher the resection, the greater the chance of vagal interruption and, if less than 75 per cent is resected, parietal cells will be left. Usually a 75 per cent resection has been accepted as standard except in old patients, or in females where less stomach is excised.

The standard 75 per cent gastrectomy abolishes the antral phase of secretion completely and includes resection of the bulk of the parietal cell mass. In addition, it may diminish or abolish the cephalic phase of secretion as determined by the Hollander test (11, 12, 13). That this is caused by interfer-

ence with the vagal fibers is indicated by several observations. For example, Smitty found that the usual Polya resection in which the left gastric artery was ligated on the wall of the stomach at the level of transection, did not produce achlorhydria but, if the lesser curvature was denuded of all tissues completely, the Polya resection was regularly followed by achlorhydria (14).

The usual result of the 75 per cent gastrectomy is to reduce acid secretions to a low level except in those patients who develop an anastomotic ulcer and in whom the pH is approximately 2.0 to 3.0 under basal conditions, or after stimulation with broth, insulin, or histamine (9).

Resection of 50 per cent or less of the stomach.—The antral phase of gastric secretion can be eliminated by a 40 per cent gastrectomy (15). Resection of the antrum also decreases the blood supply to the fundus of the stomach, and may diminish fundal function in this fashion. This action is believed to be mediated through the hypothalamus (16). However, in man, the application of 50 per cent gastrectomy or less, without vagotomy, to a large group of patients with duodenal ulcers has led to so many recurrences that the operation has been abandoned.

Vagotomy alone.—The two important effects of complete bilateral vagotomy are the elimination of the cephalic phase of gastric secretion and serious interference with normal gastric peristalsis (1, 2, 17). In addition, following vagotomy, the stomach responds to all known stimuli of HCl secretion less vigorously than it did previously (6, 18). Recent evidence has also shown that vagal stimulation may release gastrin from the antrum (19).

Early tests in dogs with total gastric pouches indicated that vagotomy decreases the total secretion by 76 per cent (20). Harkins estimated that in man with duodenal ulcer the cephalic phase accounts for 45 per cent of total secretion; 45 per cent is ascribed to the antrum, and 10 per cent to the intestinal phase (21).

Surprisingly enough, in the Heidenhain pouch dog vagotomy actually increases the amount of gastric HCl secretion (22, 23). This is due to the ineffective peristalsis resulting from vagotomy, allowing food retention in the antrum and an increased output of gastrin, which more than counterbalances the reduction in the cephalic phase of secretion. This effect is not abolished by gastroenterostomy, but is rectified by pyloroplasty (21, 24, 25).

In man, except in the presence of pyloric obstruction, vagotomy produces a reduction in volume and acidity of gastric secretion, as measured in the fasting state or after stimulation with test meals, alcohol, histamine, or psychic stimuli (8). Clinically, however, vagotomy alone is an unsatisfactory operation because it leads to poorer emptying of the stomach and must be supplemented either by gastroenterostomy or pyloroplasty, or by some type of gastric resection.

While vagotomy is believed to eliminate the cephalic phase of gastric secretion as measured by a postoperative negative Hollander test (17), there remains some doubt that all cholinergic fibers run through the vagus. In cats, cholinergic fibers are present in the sympathetic chain (26). In vagotomized

dogs with totally denervated gastric pouches, teasing occasionally produces a rise in secretion (19). In man, a positive Hollander test was observed in six patients who had had esophageal resections for cancer, and who undoubtedly had had resection of both vagi (12).

There is also the disquieting fact that although the vagi have never been shown to regenerate, cholinergic activity tends to return years after a complete vagotomy as determined by Hollander tests. Brooks & Moore studied 28 patients who had had vagotomy alone 6 to 10 years before; six showed a clear-cut early rise in free and total acid after insulin injection, and two a late rise, ascribed possibly to adrenal activity (27). Knox & West studied patients who had been treated for an anastomotic ulcer by vagotomy alone; after a 10-year period, reversal of a negative Hollander test was noted in 6 patients of 25 tested (28). On the other hand, Grimson has doubted such a reversal though some of his published protocols suggest the same trend (29). Also, Dragstedt believes that a positive Hollander test postoperatively always means that the vagotomy has been incomplete (20).

Gastric resection with vagotomy.—Total gastrectomy necessarily is accompanied by a bilateral vagotomy and gives complete control of the acid-peptic factor, even with the Zollinger-Ellison syndrome (30). Seventy-five per cent gastrectomy with vagotomy has given excellent control of the acid factor, but at an increased price from other sequelae of operation (31).

Fifty per cent gastrectomy with vagotomy was judged by Smithwick on the basis of secretory studies in 1951, to give slightly superior results to the standard 75 per cent resection except for a few instances in which the vagotomy appeared to be incomplete. This early opinion has been confirmed by others and so far has stood the test of time (9, 32). An even more conservative resection has been advocated (33); we believe the follow-up period is too short to evaluate such minimal procedures.

Gastrojejunostomy with and without vagotomy.—Measured by Heidenhain pouches in dogs, gastrojejunostomy alone will increase HCl secretion; this effect is accentuated by a high gastroenterostomy and eliminated only if the gastroenterostomy is so small that it becomes non-functional (34, 35, 36). These results are due to gastric stasis with increased antral stimulation and gastrin production, since partially digested food in alkaline duodenal juice is poured back into the stomach through the open stoma. HCl secretion in Heidenhain pouch dogs is increased by the addition of vagotomy to the gastroenterostomy (23).

In man, gastroenterostomy alone has been discarded as a method of treatment of ulcer except in essentially achlorhydric patients. After vagotomy and gastrojejunostomy secretory levels of HCl have shown less satisfactory reduction than after subtotal gastrectomy, or after 50 per cent gastrectomy and vagotomy (9).

Thus, gastroenterostomy and vagotomy is a poor operation from the point of view of HCl control but it is the safest operation for duodenal ulcer.

Pyloroplasty with vagotomy.—Pyloroplasty alone has been discarded as an

operation for ulcer. Combined with vagotomy in dogs, secretion from a Heidenhain pouch is reduced well below the normal and is far superior to vagotomy plus gastroenterostomy to control gastric HCl (25). Clinical reports are optimistic though secretory studies are not available.

Antral exclusion.—Antral exclusion studied in the Heidenhain pouch dogs increases the gastric HCl output. This effect is due to the bathing of the antral mucosa in alkaline duodenal juice and the elimination of the depressing effect of gastric HCl on the antrum or duodenum (37, 38).

In man, antral exclusion alone leads to a very high incidence of anastomotic ulcer within two to six weeks. Interestingly enough, (and here laboratory investigations are at variance witb clinical observations), secretory tests show that this operation leads to reduced acid production (12). After vagotomy is added to antral exclusion, HCl secretion is further depressed and the gastric remnant becomes unresponsive to stimulation by broth, histamine or insulin. This depression is essentially the same as that observed after 50 per cent gastrectomy and vagotomy (12).

Tubular and segmental resections.—Experimentally, using the Heidenhain pouch as an indicator, Wangensteen's tubular resection produced hypersecretion regularly; after segmental resection, pouch secretion is either diminished or approximately the same as in controls. Vagotomy added to either of these operations increased pouch secretion tremendously (39).

Since recurrent peptic ulcer is impossible in the presence of absolute achlorhydria there is a tendency to believe that the greater the degree of achlorhydria, the better the operation. There is a question in the minds of the authors as to whether or not this attitude is entirely correct. Certainly, some undesirable effects may be ascribed to achlorhydria. First, the growth of various Gram-negative bacilli, which customarily inhabit only the colon, may occur in the small intestine or in the stomach in the presence of achlorhydria. The danger of this complication has been stressed by Kunz & Waddell who have found nine instances of Salmonella infection in such patients; one of these was fatal (40). They believe that such infections may account for many of the undiagnosed diarrheas that follow gastric operations. Secondly, hydrochloric acid has definite metabolic benefits. It promotes fat absorption (41) and, by reducing ferric iron to ferrous, which is more easily absorbed, promotes iron absorption. In the third place, it is known that the incidence of cancer of the stomach is higher in patients with achlorhydria than in normals (42). It would therefore seem reasonable that the late follow-up of patients operated on for ulcer might show an increased incidence of cancer over that expected in the normal. While there is a good deal of difficulty in these follow-ups, suggestive evidence has appeared that this may be true (43).

PEPSIN

Ulcer patients secrete three times as much pepsin as controls. It may be measured by the proteolytic activity of aspirated gastric contents, or by

measurement of pepsinogen in the blood or, as its excretory produce in the urine, uropepsin. The early belief that a constant fraction (about 1 per cent) of the secretion of the chief cells would be excreted into the blood indicated a very convenient linear determination of pepsin by pepsinogen assay (44). However, it is now far from certain that a constant amount of pepsin is secreted into the blood stream, and many of the observed clinical discrepancies are easier to explain by the theory that variations in gastric mucosa actually may block intragastric secretion at times and promote back flow into the blood stream.

Uropepsin measured by Gray and his co-workers on normal subjects ranged from 1200 to 3800 units with a mean output of 2300 units and a standard deviation of 1700 units. The mean output with duodenal ulcer was 8487 units; for gastric ulcer, 6000 units, and gastric cancer, 980 units, and absent in pernicious anemia. In a group of 10 patients treated by subtotal resection for ulcer, the preoperative mean excretory level was 7010 units, and postoperative, 2792 units. After vagotomy the mean level fell only from 4923 to 4196 units. In seven patients with an anastomotic ulcer the mean level was 10,126 units (45).

It may be concluded from this and other studies that uropepsin excretion is abolished by total gastrectomy, and reduced by subtotal resection. There is some difference of opinion as to whether or not it is influenced by vagotomy. It had been hoped that an approximately linear relationship could be attained between the uropepsin level and the anatomic extent of gastric resection for ulcer. However, further studies have shown that the range of normal is so great, that the test is less valuable than early reports had indicated.

Spiro, Ryan & Jones believe that blood pepsin assay is superior to uropepsin studies, and that patients with high postoperative blood pepsin levels may well be regarded as candidates for further ulcer complications. Thus, the mean level of 24 asymptomatic patients after subtotal gastrectomy was 250 units, and 13 patients with symptoms, 450 units. It also was of interest that the median postoperative level was 336 units and, of non-operated patients with duodenal ulcer, 580 units (46).

Hoar & Browning, in a study of plasma pepsinogen, found a mean level of 689 units in patients with duodenal ulcer, 705 with gastric ulcer, 314 after subtotal gastrectomy, and 147 after total gastrectomy (47). This last figure suggests the possibility of extra gastric pepsinogen and could contribute further doubt about the value of this study as a diagnostic test.

Mucoprotein

Gastric mucin is secreted by glands that occur throughout the stomach but are predominantly in the fundus. Relatively few studies of mucin in the normal postoperative stomach have been published except by Glass and his associates (48, 49). They believe that glandular mucoprotein is important, not only because it contains a protective substance which coats gastric mu-

cosa, but also because it appears to be the main source of the intrinsic factor activity in the gastric juice of man. They have found that after glandular mucoprotein produced from gastric juices of normal individuals is added to small oral doses of vitamin B_{12} that are ineffective by themselves, a sharp hematopoietic response is obtained.

Glandular mucoprotein is, of course, eliminated after total gastrectomy. It is almost normal after distal subtotal resection since most of the mucus-secreting glands are in the fundus. Mersheimer *et al.* found that in the intact stomach hyperglycemia produced a moderate rise of mucous secretion. Histamine administration in vagotomized patients and in the dog also causes a rise in secretion of mucus, and the mucus-secreting cells appear normal histologically after vagotomy. Mucin secretion, therefore, apparently can be stimulated by vagal and hormonal pathways (49).

Hormones

The antral mucosa secretes gastrin, which stimulates the parietal cells in the corpus to secrete acid (1, 46, 47, 50, 51) and probably a second hormone that depresses HCl secretion (1, 52, 53, 54). No hormone is known to exist in the proximal portion of the stomach. Gastrin formation normally is stimulated by application of alkaline or neutral foods to antral mucosa or by constant mechanical stimulation of the antrum. It may be depressed by local anesthesia or by the application of acid to the antral mucosa (1, 53). This depressing effect is probably caused by a second hormone as indicated by the recent cross-circulation experiments of DuVal (54).

Complete resection of the antrum requires at least 40 per cent gastrectomy. Antrectomy will eliminate these two hormones, of which the secreting hormone is by far the more important.

Experimentally, in dogs with Pavlov or Heidenhain pouches, resection of the antrum produces a striking *fall* in the secretion of HCl, averaging about 84 per cent of the total (1). In man with duodenal ulcer, 45 per cent of gastric HCl is estimated to be duc to the antral phase of secretion (21).

DEFECTS OF ABSORPTION

These defects are caused chiefly by loss of reservoir function or capacity of the stomach and, secondarily, to more rapid transit time through the small intestine. Other factors are of importance; for example, the dumping syndrome may accentuate a loss of appetite and lead to a deficient intake of food. Consideration will be given to changes in carbohydrate metabolism, including the dumping syndrome, fat, protein, vitamins, minerals, weight, and growth, and then to operations designed to retain reservoir function.

Carbohydrates

After gastrectomy the absorption of carbohydrates is more rapid than in the normal because of more rapid exposure of carbohydrates to the jejunal mucosa. A maximum blood sugar concentration was reached in 30 to 60 min.

after a test meal in dogs which had had total gastrectomy as compared to 120 min. in the normal. The hyperglycemia leads to increased insulin production and a secondary hypoglycemia is more marked after gastrectomy than in the normal. There apparently is no difference between normal and gastrectomized dogs in the rate of utilization of absorbed sugar as determined by intravenous glucose tolerance tests (55).

Carbohydrate balance studies are not informative since any excess carbohydrate in the diet is utilized by bacteria in the bowel in both normal and postoperative patients.

Dumping syndrome.—The most important physiological abnormality after gastrectomy, the dumping syndrome, is closely related clinically to carbohydrate metabolism, and therefore will be considered here.

This is a syndrome characterized by many symptoms. They are, according to Weidner *et al.* in the order of their frequency, fullness, desire to lie down, borborygmi, nausea, eructation, diarrhea, gastric distress, weakness, dizziness, drowsiness, nervousness, restlessness, palpitation, irritability, perspiration, vomiting, cramping abdominal pain, sense of warmth, headache, thirst, sighing, urge to defecate, and chilliness (56). Though it is most common after high Billroth II resections, it may occur after all types of gastric operations, and is demonstrated after the ingestion of hyperosmolar carbohydrate solutions, such as sugar and starch. It appears about 10 min. after ingestion and disappears in 30 to 60 min. (56, 57).

The incidence of this disorder is estimated variously after gastrectomy from 3 to 75 per cent depending entirely upon the diagnostic criteria employed. In our experience, major symptoms of dumping occur after 3 per cent of all gastrectomies and minor symptoms in about 20 per cent. According to Kiefer, 10 years after subtotal gastrectomy, 86 per cent of patients had had no dumping symptoms, 5.7 per cent had had temporary symptoms, 3.9 per cent had had mild but persistent symptoms, and 4.3 per cent had important and persistent symptoms (58). To establish some uniformity of definition, a number of investigators employ a standard test meal, such as 150 cc. of 50 per cent glucose as the challenging dose to tell whether or not dumping exists; it should be noted that this is a much more severe test than the patient ordinarily gives himself in his usual diet.

Physiologic changes that occur with this syndrome provide basis for several theories concerning its origin. Important physiologic changes include: (*a*) reduction in plasma volume. Roberts, Randall *et al.* demonstrated a definite fall of plasma volume with the onset of dumping symptoms (59). They believe that the dilution of hypertonic solutions in the jeunum results in such a shift of extracellular water and perhaps electrolytes, that there is an acute drop in blood volume. This drop in plasma volume which may be as great as 1000 cc., though usually is from 300 to 750 cc., may be corrected by the rapid intravenous administration of water with elimination of symptoms. This drop in plasma volume has been confirmed by many investigators (60, 61), though not all agree (62). Although this drop is significant, it has

been noted that usually it is not of the magnitude to produce symptoms in a normal person. Furthermore, the variation of hematocrit is not striking (63). (*b*) Electrocardiographic changes may occur that are similar to those observed by hypokalemia. There is lengthening of the QAS segment with depression of the T-waves (61, 64). (*c*) The electroencephalogram shows definite dysrhythmias at the height of the dumping symptoms (61). (*d*) Skin temperatures increase 3 to 5 degrees with dumping symptoms, while peripheral blood flow increases from $1\frac{1}{2}$ to 2 times that observed in the controls (61). (*e*) Serum potassium shows a mild fall but this occurs 75 to 80 min. after the onset of the dumping symptoms. This fall occurs simultaneously with the hypoglycemia and probably is attributable to glycogen deposition in the liver, potassium being bound in these cells (65, 66). (*f*) Other objective manifestations include tachycardia, elevation of blood pressure, increased small intestinal intraluminal pressure and motility, decreased plasma phosphate, increased urinary excretion of uric acid, decreased circulating eosinophils, hyperglycemia, decreased cardiac output, and a rise in renal blood flow (64, 67, 68). (*g*) Rapid emptying of the stomach may be demonstrated by x-ray studies in most of the patients. In fact, many authors believe that this test should be used to separate the dumping syndrome from other postgastrectomy abnormalities, suggesting a return to the original definition of the term by Mix (69, 70).

Krieger, Abbott *et al.* believe that there is complete correlation between rapid gastric emptying and the dumping syndrome (70). However, this impression does not agree with many other observations. Certainly, many patients after Billroth II gastrectomies demonstrate rapid emptying of the stomach without dumping symptoms; in fact, in nearly all instances barium passes immediately into the small intestine after it is given by mouth (71).

Furthermore, interesting data by Henshaw *et al.* indicate that there is some individual reaction to hypertonic glucose. When 150 cc. of 50 per cent glucose are given by mouth to normal men, no symptoms result. When an intestinal tube was passed in 30 patients with duodenal ulcer, and the same test meal injected rapidly into the jejunum, two developed severe dumping symptoms, 10 moderate, 9 mild, and 9, none at all. Postoperatively, there was very close agreement with the preoperative test on repetition of the test meal (72).

At present it would seem that the theory advanced by Roberts and Randall is the most likely explanation of the dumping syndrome, though there is no explanation of the variability of the jejunal response in various patients to the challenging meal.

Fats

Absorption of fat is impaired markedly by total gastrectomy and, to a lesser degree, by the subtotal. In dogs, fat loss in controls after gastroenterostomy was 4.9 per cent, after segmental gastrectomy, 6.4 per cent and, after subtotal gastrectomy with Billroth I anastomosis, 10.6 per cent (73).

Losses after Billroth II anastomosis were greater and depended on the extent of the resection; with 50 per cent resection it was 6 per cent; two-thirds resection, 20 per cent; 75 per cent resection, 28 per cent; and, after total gastrectomy, 32 per cent (74).

Some authorities believe that in man careful studies are not available as yet to prove that the various anastomotic procedures modify fat absorption significantly (41, 75). However, Ellison found that both the extent of resection and type of anastomosis were important. A 70 per cent resection and Billroth II anastomosis was associated with 80 per cent absorption; a 75 per cent resection, 71 per cent; and an 85 per cent resection, 61 per cent. The over-all figures for Billroth I were 79 per cent; Billroth II, 70 per cent; Billroth II with vagotomy, 65 per cent (76).

Since various authors have established different criteria for fat absorption in the normal, the reported abnormalities after resection likewise will vary. Lawrence *et al.* using 92 per cent absorption as the lower limit of normal, found that 22 of 25 patients had abnormal fat absorption after total gastrectomy (41). Everson, using less vigorous criteria, estimated that 21 of 33 patients had abnormal curves after total gastrectomy (77). In general, after total gastrectomy, about 20 per cent of dietary fat is excreted, compared with 5 per cent in the normal. After subtotal gastrectomy, the range is closer to normal; in Lawrence's 17 cases the median value was 90 per cent and 11 had normal absorption while impairment was noted in 23 of 36 cases analyzed by Everson.

Lawrence *et al.* emphasize the fact that this steatorrhea is much less severe than that which occurs with sprue, after massive intestinal resections, or with some pancreatic disease, and tends to diminish with the passage of time. They believe it actually is of little clinical significance, and there is relatively poor correlation between the actual ability to maintain body weight and the degree of steatorrhea. This fact is disputed by Hayes who noted that in the normal patient delay in gastric emptying following the administration of fat (78). This delay persists after gastroenterostomy but does not after resection of the antrum and pylorus (78, 79). To prevent the rapid development of a hyperosmolar solution in the proximal jejunum, a diet in the caloric ratio of carbohydrate, 1, protein 1, and fat, 5, is recommended; patients treated in this fashion had complete relief of dumping symptoms and weight restoration to near normal (78).

Proteins

Protein absorption is decreased after total gastrectomy and, to a lesser degree, after subtotal. In control dogs, 14 per cent of nitrogen was excreted and after segmental resection, 12.6 per cent; after Billroth I resection, 19.3 per cent; and after Billroth II, 24.4 per cent (73).

Reports of 27 metabolic studies carried out in 14 individuals were collected by Everson, using 2 gm. of fecal nitrogen loss as the upper limit of

normal. There was evidence of some impairment of protein absorption in 6 of the 14 (77).

Lawrence *et al.* found that the total nitrogen excretion at a zero balance was 168 mg. per kg. of body weight, per day, after total gastrectomy. This was in contrast witb the normal excretion of 112 mg. kg. per day as calculated by von Hoesslin (80) and by Beattie *et al.* (81). The increased nitrogen excretion can be ascribed entirely to an increased stool nitrogen. Consequently, the usual normal intake of protein ordinarily will be insufficient for the patient who has had a total gastrectomy.

An increase in carbohydrate intake led to a decreased absorption of nitrogen in 9 of 10 patients after total gastrectomy (41). The mean decrease in absorption in this group was 9.4 per cent. After subtotal gastrectomy no significant change was noted with variations of carbohydrate intake. Notwithstanding the decreased absorption ascribed to it, increase in carbohydrate intake may increase nitrogen retention, possibly because of the increased caloric intake.

Additional hydrochloric acid given prior to meals (25 cc. of 0.1 *N* HCl) led to a sligbt decrease in absorption of proteins (5.7 per cent) (41).

Abnormal Loss of Vitamins

After operation, fat-soluble vitamins may be absorbed poorly, secondary to increased loss of fat in the feces. After gastrectomy, vitamin A absorption was found to be reduced to 31 per cent of normal, though administration of wine increased the absorption rate 125 per cent (82). Vitamin D also may be absorbed poorly after gastrectomy (*vide infra*). Lack of absorption of vitamin in B_{12} will be discussed later. (See Anemias.)

Minerals

Postoperatively, minerals may be absorbed poorly because of more rapid transit time tbrough the intestine. Iron loss may contribute to a secondary anemia (70). An excessive loss of potassium secondary to diarrhea following total gastrectomy has been reported to lead to sudden death (83). Since calcium loss is the most important it will be considered in more detail.

Osteomalacia was found by Melick & Benson in two patients after subtotal gastrectomy; five additional cases are described in the literature (84). Bone pain and low serum calcium and pseudofractures led to the diagnosis. Inadequate absorption of vitamin D was considered to be the primary cause. Malabsorption of fat was demonstrated in both cases. Calcium levels responded to large doses of vitamin D and calcium given by mouth.

Other factors such as inadequate intake therefore are also important. In addition, growth may increase the demands for calcium. Thus, Bussabarger *et al.* found that total gastrectomy in growing puppies receiving vitamin D by mouth produced severe osteoporosis with fractures and bowing (85).

Steatorrhea from any cause is likely to lead to osteomalacia because of

the precipitation of calcium soaps in the gut reducing the amount available for absorption and, as noted above, steatorrhea is not uncommon after gastrectomy. Baird & Oleesky observed that this disease takes at least 2 years to develop after subtotal gastrectomy. They have observed four patients and believe there must be many more with osteomalacia. They advise 100,000 to 200,000 units of vitamin D daily, together with 5 to 10 gm. of calcium lactate (86).

Weight Loss or Gain

Weight loss is not common after operations on the stomach unless some type of resection is performed. Under these circumstances the loss varies in general directly with the amount of stomach resected and it is higher after Billroth II than after Billroth I anastomosis. In similar operations weight losses tend to be more severe in women.

The amount of expected postoperative weight loss can be estimated preoperatively. According to Zollinger & Ellison these patients may be divided into three groups: (*a*) those whose preoperative weight was equal to or about their ideal weight; they tend to maintain a satisfactory weight after gastrectomy: (*b*) those below ideal weight at the time of operation, though at one time they had had an ideal weight; only about one-third of this group returned to normal postoperatively: (*c*) those who had never attained an ideal weight preoperatively never gained it postoperatively. No laboratory tests have been found that can give a better prognosis than these clinical observations (87).

Many reports have been made of weight losses after operation but only one will be cited here. Ten years after subtotal gastrectomy weight was found to be normal in 46 per cent of patients, while 34 per cent weighed at least 10 pounds less than normal, and 20 per cent weighed at least 10 more than normal (58).

Growth

Growing puppies appear to tolerate subtotal gastrectomy without interference with growth (88). Relatively few observations are available in man since gastric resection is very uncommon in children, though a few reports indicate that normal growth continues despite subtotal gastrectomy (89), and this is in accord with our personal observations.

Operations Designed to Retain the Gastric Reservoir

This objective has been attained in part by several methods—use of the Billroth I rather than the Billroth II anastomosis, by retention of the pylorus at the time of the gastric resection, and by the formation of a small stoma after Billroth II resection.

Reservoir function generally is maintained better after Billroth I than after Billroth II resection: this, in part, may be the result of the less extensive resection that accompanies a Billroth I. Very interesting radiographic studies

have been made by Liljedahl *et al.* (71). After Billroth I resection the motility pattern of the stomach is found in all essentials to resemble that seen in healthy subjects. A formation of a functional pylorus usually can be observed. This pseudosphincter is not seen after a Billroth II resection.

In certain instances a normal pylorus may be retained. Friesen & Rieger believe that retention of a normal pylorus will essentially eliminate the dumping syndrome. Dogs that had sleeve-type resections without vagotomy demonstrated slower gastric emptying and a significantly smaller loss of plasma volume following hypertonic glucose test meals than did those dogs in which the pylorus was excised or was non-functional. A small group of patients showed similar results (66).

A few surgeons now believe that a small gastroenteric stoma, about $1\frac{1}{2}$ cm. in diameter, is the key to maintenance of the normal gastric reservoir, and abolition of the dumping syndrome after Billroth II anastomosis (70, 90, 91). This matter is still *sub judice.*

ANEMIAS

Several types of anemia have followed gastrectomy-megaloblastic anemia, hypochromic anemia due to reduced absorption of iron, and hypochromic anemia due to blood loss.

Megaloblastic anemia.—This is believed to occur regularly in all patients who have had total gastrectomy, and in about 1 per cent of the patients who have had subtotal gastrectomy, provided they have lived for over 3 years from the date of operation and have not taken proper replacement therapy (92, 93, 94). The development of megaloblastic anemia may be due to a number of reasons. There may be, for example, a loss of intrinsic factor, due to a high or total gastric resection. On the other hand, if the normal amount of intrinsic factor is present, the extrinsic factor (Vitamin B_{12}) may be absent in the oral intake, not absorbed (as occurs after extensive resection of the ileum), or destroyed before absorption, as in the presence of blind intestinal loops or diverticulosis of the intestine.

Halsted *et al.* found that normal persons excrete 19 to 57 per cent of orally administered vitamin B_{12} in the feces, compared with an excretion rate of 84 to 100 per cent in patients with pernicious anemia and 75 to 100 per cent in those who had total gastrectomy. These high excretions in the last two groups can be restored to normal if intrinsic factor is supplied to the patient (95).

After total gastrectomy, a patient who is receiving no replacement therapy will use up his vitamin B_{12} stores at the rate of 1 μg. per day. Since the total body reserve is 1000 to 2000 μg., approximately three to four years will be required before anemia can develop. McLean believes, from a careful study of 13 patients with total gastrectomy, that a megaloblastic anemia will occur inevitably at that time unless replacement therapy has been given.

In so far as prophylaxis or treatment of the anemia is concerned, monthly administration of 50 μg. of intramuscular vitamin B_{12} appears to be ade-

quate except when neurological symptoms are present and the dose then should be larger. Liver extract has been used as an alternative source of B_{12}. Folic acid therapy has been found necessary in some reported cases from England and Italy, in addition to vitamin B_{12}; though folic acid will ameliorate the blood picture, it will not prevent neurological deficits and it should not be used without vitamin B_{12} (96).

Megaloblastic anemia also occurs after subtotal distal gastrectomy but usually is not suspected until it is found that the anemia fails to respond to iron. McLean found that only 9 of 1550 patients developed this type of anemia. It was of interest that all of these patients had either gastric ulcer or cancer and that a pouch of normal fundus consisting of more than 10 per cent of the total stomach appeared capable of producing sufficient intrinsic factor to prevent vitamin B_{12} deficiency (92).

Hypochromic anemia due to iron deficiency.—Low serum iron values are common after total, and occasionally after partial gastrectomy. As a consequence, hypochromic anemia may develop, though decreased iron values may be found without overt signs of anemia. Birnbaum *et al.* noted that serum iron levels tended to be lower postoperatively in females than in males (97).

Lyngar has investigated tbe frequency of this type of anemia. A series of patients with uncomplicated gastric ulcer were taken as controls. Their blood picture did not deviate significantly from that of normal individuals. It was certain several years after extensive subtotal gastrectomy that at least 43 per cent of the women and 10 per centof the men had become anemic. The increased frequency in females was observed in the menstruating age group (98).

He also found that in all cases it was possible to cure the anemia by the administration of iron. Usually it could be given orally though occasionally parenteral therapy was necessary. It seems probable that iron absorption is inadequate in these patients because of relative achlorhydria and more rapid transit time through the intestine. More than a third of these patients showed sideropenic epithelial symptoms. Dyspeptic symptoms, somewhat suggestive of the dumping syndrome, were relieved as the anemia was cured (98).

Hypochromic anemia due to blood loss.—Careful examination should be carried out in all patients found to be anemic after gastrectomy. Occasionally an anastomotic ulcer will be discovered, or blood loss from other lesions may be found.

CHANGES SECONDARY TO STASIS OR REFLUX

Stasis secondary to too small a gastroenteric stoma, or to a high gastroenterostomy, or to a vagotomy unaccompanied by a drainage procedure, leads to retention of food. Unless all of the antrum has been resected, an increased output of gastrin occurs so that the HCl output is increased. Hence,

too small a stoma may lead not only to the usual symptoms of obstruction, but also may contribute to ulcer recurrence (99).

Reflux of alkaline contents from the proximal loop into the stomach is said by Schindler to occur regularly after Billroth II anastomosis and most uncommonly after Billroth I, and to cause a specific postoperative gastritis (100). Other gastroscopists have failed to share his excitement about this particular type of gastritis, nor have they been able to demonstrate it by biopsy (101). Resected Billroth II anastomoses rarely show any microscopic evidence of gastritis.

On the other hand, particularly when the afferent loop is long, it may fill with bile and pancreatic secretions and empty irregularly into the stomach and produce occasional attacks of vomiting, in which the vomitus is copious but not mixed with food. This has been termed the "afferent loop syndrome" and is a comparatively rare complication of gastrectomy (102). It has been suggested that the development of an abnormal bacterial flora in the long loop may be an important factor in abnormalities of absorption (103).

SECONDARY EFFECTS ON OTHER ORGANS

A relationship of operations on the stomach to other organs is suggested by fragmentary data. Since hydrochloric acid applied to the sphincter of Oddi causes stimulation of pancreatic secretions, any effective operation for ulcer should diminish pancreatic activity (104). Likewise, vagotomy decreases pancreatic secretions (105).

Nearly all observations on the intestine are confined to motility changes. The decreased small bowel transit time after gastrectomy, and increased activity of the colon result from rapid emptying of the stomach and the small intestine. Increased enzymatic activity of the small intestine has been demonstrated after partial gastrectomy in dogs (106).

Variations in blood flow in various organs have been shown to occur with the dumping syndrome (67). Interestingly, a reduction in the incidence of coronary thrombosis in patients who have had operations for ulcer has been reported (107).

RECURRENT ULCERATION

Factors causing recurrent ulceration are not entirely clear. Such an occurrence always suggests the possibility of the Zollinger-Ellison syndrome. Though an uncontrolled acid-peptic factor must be present, this is difficult to demonstrate in many instances (63). Acid levels tolerated by some patients cannot be tolerated by others.

The location of the gastrointestinal anastomosis is of great importance; thus, resistance to acid-peptic digestion is highest in the cells of the gastric mucosa, and lower progressively in intestine, colon, and esophagus. Anastomosis of isolated gastric pouches in the dog to progressively more distal

quate except when neurological symptoms are present and the dose then should be larger. Liver extract has been used as an alternative source of B_{12}. Folic acid therapy has been found necessary in some reported cases from England and Italy, in addition to vitamin B_{12}; though folic acid will ameliorate the blood picture, it will not prevent neurological deficits and it should not be used without vitamin B_{12} (96).

Megaloblastic anemia also occurs after subtotal distal gastrectomy but usually is not suspected until it is found that the anemia fails to respond to iron. McLean found that only 9 of 1550 patients developed this type of anemia. It was of interest that all of these patients had either gastric ulcer or cancer and that a pouch of normal fundus consisting of more than 10 per cent of the total stomach appeared capable of producing sufficient intrinsic factor to prevent vitamin B_{12} deficiency (92).

Hypochromic anemia due to iron deficiency.—Low serum iron values are common after total, and occasionally after partial gastrectomy. As a consequence, hypochromic anemia may develop, though decreased iron values may be found without overt signs of anemia. Birnbaum *et al.* noted that serum iron levels tended to be lower postoperatively in females than in males (97).

Lyngar has investigated the frequency of this type of anemia. A series of patients with uncomplicated gastric ulcer were taken as controls. Their blood picture did not deviate significantly from that of normal individuals. It was certain several years after extensive subtotal gastrectomy that at least 43 per cent of the women and 10 per cent of the men had become anemic. The increased frequency in females was observed in the menstruating age group (98).

He also found that in all cases it was possible to cure the anemia by the administration of iron. Usually it could be given orally though occasionally parenteral therapy was necessary. It seems probable that iron absorption is inadequate in these patients because of relative achlorhydria and more rapid transit time through the intestine. More than a third of these patients showed sideropenic epithelial symptoms. Dyspeptic symptoms, somewhat suggestive of the dumping syndrome, were relieved as the anemia was cured (98).

Hypochromic anemia due to blood loss.—Careful examination should be carried out in all patients found to be anemic after gastrectomy. Occasionally an anastomotic ulcer will be discovered, or blood loss from other lesions may be found.

CHANGES SECONDARY TO STASIS OR REFLUX

Stasis secondary to too small a gastroenteric stoma, or to a high gastroenterostomy, or to a vagotomy unaccompanied by a drainage procedure, leads to retention of food. Unless all of the antrum has been resected, an increased output of gastrin occurs so that the HCl output is increased. Hence,

recurrence (119), a 10 per cent recurrence rate at five years would seem to be a fairly close approximation to what is observed elsewhere. Vagotomy plus pyloroplasty has not had a long follow-up. Weinberg has reported a 5 per cent estimated recurrence rate (120). (*g*) Antral exclusion operations: nine recurrences after 154 operations including antral exclusion plus vagotomy have been recorded by Waddell after a short follow-up period (12). (*h*) Segmental resections: Wangensteen had discarded tubular resection because of the higher recurrence rate; he has so far encountered no recurrent ulcers with the segmental resection (121). Ferguson noted a possible recurrent crater in 4 per cent of patients though ulcer-type pain was noted in 16 per cent three months to five years after segmental gastrectomy (122).

DEATHS

Operative mortality depends upon many factors, a detailed discussion of which is beyond the scope of this paper. The complications of acute perforation and massive hemorrhage carry a relatively high mortality. Elective procedures furnish the most acceptable basis for comparison of various operations. In this category vagotomy combined with gastroenterostomy, or pyloroplasty carries a mortality of 0.5 to 1.5 per cent, while the average mortality for elective gastric resection for duodenal ulcer throughout the country is probably about 3 per cent. There is, in addition, a late mortality due to recurrent ulcer. Moore found no late mortality from ulcer three to seven years following subtotal gastrectomy (114). Kiefer found an additional late mortality of 2.1 per cent due to recurrent ulceration within 10 years of the date of the primary resection (58).

In conclusion, it is well to note that physiologists and clinicians owe a great debt to previous generations of investigators. Though nearly all references in this review would indicate that most of the knowledge of the physiology of the postoperative stomach has been acquired recently, important principles had been established over 50 years ago. Thus, Cannon's observations on the motility and method of emptying of the stomach after gastro-enterostomy are as fresh now as when they were published in 1909 (123). Joslin & Goodall's two determinations of 18.7 per cent, and 31.2 per cent of loss of ingested fat in the feces after gastroenterostomy in 1907 (124), are in the range expected today. While the past 50 years have led to an accumulation of much additional information, we still await, as they did then, an operative procedure that can correct peptic ulcer and produce a stomach as satisfactory as Nature has provided in the normal man.

levels of the gastrointestinal tract was accompanied by a progressively higher incidence of anastomotic ulceration (108). Similarly, after Billroth II anastomosis the more distal the anastomosis the greater is the incidence of anastomotic ulcer (109). This is probably due to a progressive decrease in pH and buffering capacity of small bowel contents as the distance from the pylorus is increased (110). On the other hand, if unneutralized gastric contents are dripped upon isolated segments, it is found that the stomach is relatively resistant, the esophagus is very sensitive, and the various portions of the small intestine nearly equally sensitive to digestion, except for the most proximal portion of the duodenum which is more resistant because of the mucous secretion from Brunner's glands (111).

For this reasoo, it would seem that ulceration in the Billroth I anastomosis would be less likely to recur than in Billroth II. However, the opposite is the case. This is explained by the protection afforded the Billroth II anastomosis by the continued stream of alkaline duodenal contents that ruo over it. The importance of this reflux protection of the anastomosis has been furnished by withdrawal of duodeoal contents by suction after Billroth II gastrectomy. Very high gastric acidity was found that returned to normal with closure of the duodenal fistula (112, 113).

Though the factors that produce recurrent ulcer are not understood, the incidence following various operations is fairly uniform. Representative rates after various operations are as follows: (*a*) Total gastrectomy: 0 per cent; (*b*) 75 per cent gastrectomy and vagotomy: 0.5 per cent (31); (*c*) subtotal gastrectomy: 3 to 7 years after operatioo Moore *et al.* found an incidence of 3.2 per cent of anastomotic ulcer (114). In a minimal four-year follow-up Wallensten found that in men ulcer recurred after a Billroth I procedure for gastric ulcer in 6.1 per cent, and after the Billroth II for gastric ulcer in none; for duodenal ulcer the comparable figures were 11.7 and 4.2 per cent. In women, the recurrence rate for all ulcers was 3.4 per cent after the Billroth I and 0 after the Billrotb II operations (115). Kiefer, in a 10-year follow-up found the incidence of recurreot ulcer after subtotal gastrectomy for duodenal ulcer was 13.4 per cent; for gastric ulcer, 6.2 per cent; and stomal ulcer, 17.7 per cent (58). (*d*) Fifty per cent gastrectomy and vagotomy: according to Herrington, the incidence of recurrent ulcer is below 0.5 per cent, ooly 13 instances of recurreoce baving been reported in a total of 3052 patients who have had this operation (32, 116). The follow-up period, however, is much less than that noted with subtotal gastrectomy. (*e*) Posterior gastroenterostomy: recurrence figures vary widely, depending particularly on the age of the patient, from 15 to over 50 per ceot. Johnson found an over-all recurrence rate of 52 per cent in patients followed for 5 to 10 years who were operated on below age 35 (117). (*f*) Vagotomy plus gastroenterostomy or pyloroplasty: vagotomy alone has led to recurrent gastric ulceration, due probably to increased antral stimulation (118). The increasing passage of time shows more frequent recurrence after vagotomy and posterior gastroenterostomy. Though Grimson still reports only a 2 per cent incidence of

42. Wangensteen, O. H., *Cancer of Esophagus and Stomach* (Am. Cancer Soc., New York, 1951)
43. Krause, U., *Acta Chir. Scand.*, **114**, 341 (1958)
44. Janowitz, H. D., and Hollander, F., *J. Appl. Physiol.*, **4**, 53 (1951)
45. Gray, S. J., Ramsey, C. G, and Reifenstein, R. W., *New Engl. J. Med.*, **251**, 835 (1954)
46. Spiro, H. M., Ryan, A. E., and Jones, C. M., *New Engl J. Med.*, **253**, 261 (1955)
47. Hoar, C. S., Jr., and Browning, J R, *New Engl. J. Med.*, **255**, 153 (1956)
48. Glass, G. B. J., Mersheimer, W. L., and Svigals, C. S., *Arch. Surg.*, **62**, 658 (1951)
49. Mersheimer, W. L., Glass, G. B. J., Speer, F. D., Winfield, J. M., and Boyd, L. J., *Ann. Surg.*, **136**, 668 (1952)
50. Edkins, J. S., *Am. J. Physiol.*, **34**, 133 (1906)
51. Woodward, E. R., Bigelow, R. R., and Dragstedt, L. R., *Proc. Soc. Exptl. Biol. Med.*, **68**, 473 (1948)
52. Harrison, R. C., Lakey, W. H., and Hyde, H. A., *Ann. Surg.*, **144**, 441 (1956)
53. Woodward, E. R., Robertson, C. R., Fried, W., and Schapiro, H., *Gastroenterology*, **32**, 868 (1957)
54. DuVal, M. K., Jr., and Price, W. E., *Ann. Surg.*, **152**, 410 (1960)
55. McCorkle, H. J., and Harper, H. A., *Ann. Surg.*, **140**, 467 (1954)
56. Weidner, M. G., Jr., Scott, H. W., Jr., Bond, A. G., and Shull, H. J., *Gastroenterology*, **37**, 188 (1959)
57. Ferguson, L. K., *Surg. Clin. North Am.*, **35**, 1693 (1955)
58. Kiefer, E. D., *Gastroenterology*, **37**, 434 (1959)
59. Roberts, K. E., Randall, H. T., Farr, H. W., Kidwell, A. P., McNeer, G. P., and Pack, G. T., *Ann Surg.*, **140**, 631 (1954)
60 Amdrup, E., and Jorgensen, J. B., *Acta. Chir. Scand.*, **112**, 294 (1957)
61. Henshaw, D. B., Jorgenson, B. J., Davis, H. A., and Stafford, C. E., *Arch. Surg.*, **74** 686 (1957)
62. Webber, B. M., Bender, M. A., and Moore, G. E., *New Engl. J. Med.*, **256**, 285 (1957)
63. Brooks, J. R., and Moore, F. D., *New Engl. J. Med.*, **260**, 20 (1959)
64. Peddie, G. H., Jordan, G. L., Jr., and DeBakey, M. E., *Ann. Surg.*, **146**, 892 (1957)
65. Smith, W. H., *Lancet*, **II**, 745 (1951)
66. Friesen, S. R., and Rieger, E., *Ann. Surg.*, **151**, 517 (1960)
67. Morris, C. C., Jr., Greenfield, L. J., Jordan, G. L., Jr., Peddie, G. H., Gordon, J. R., and DeBakey, M. E., *Ann. Surg.*, **150**, 90 (1959)
68. Machella, T. E., *Ann. Surg.*, **130**, 145 (1949)
69. Mix, C. L., *Surg. Clin. North Am.*, **2**, 617 (1922)
70. Abbott, W. E., Krieger, H., Levey, S., and Bradshaw, J., *Gastroenterology*, **39**, 12 (1960)
71. Liljedahl, S. O., Mattsson, O., Pernow, B., and Wallensten, S., *Acta Chir. Scand.*, **117**, 206 (1959)
72. Henshaw, D. B., Joergenson, E. J., Stafford, C. E., *Arch. Surg*, **80**, 738 (1960)
73. Everson, T. C, *Surgery*, **36**, 525 (1954)
74. Everson, T C, *Surgery*, **42**, 12 (1957)
75. French, A. B., Pollard, H. M., and Ratner, J. T, *Incidence of Postgastrectomy Malabsorption* (Am. Gastroenterological Assoc., April, 1960; not yet published)
76. Ellison, E. M., *Surgery*, **36**, 536 (1954)
77. Everson, T. C., *Intern. Abstr. Surg.*, **95**, 209 (1952)
78. Hayes, M. A., *Surgery*, **37**, 785 (1955)
79. Waddell, W. R., and Wang, C. C., *Ann. N. Y. Acad. Sci.*, **56**, 83 (1952)
80. von Hoesslin, H, *Arch. Hyg.*, **88**, 147 (1919)
81. Beattie, J., Herbert, P. H, and Bell, D J., *Brit. J. Nutrition*, **1**, 202 (1948)
82. Althausen, T. L., Uyeyama, K., and Loran, M. R., *Gastroenterology*, **38**, 942 (1960)
83 Sensenig, D. M., and Campbell, R. E., *Ann. Surg.*, **145**, 81 (1957)
84. Melick, R. A., and Benson, J. A., Jr., *New Engl. J. Med.*, **260**, 976 (1959)
85. Bussabarger, R. A., Freeman, S., and Ivy, A. C., *Am. J. Physiol.*, **121**, 137 (1938)
86. Baird, I. M., and Oleesky, S., *Gastroenterology*, **33**, 284 (1957)
87. Zollinger, R. M., and Ellison, E. H., *J. Am. Med. Assoc.*, **154**, 811 (1954)
88. Thompson, J. A., Bonta, J. A., and Clatworthy, H. W., *Surg. Forum, Proc. Congr. Am. Coll. Surgeons*, **6**, 297 (1955)
89. Green, T. H., Jr., and Hendren, W. H., III, *New Engl. J. Med.*, **262**, 118 (1960)
90. Zollinger, R. M., *Arch. Surg.*, **80**, 750 (1960)
91. McCaughan, J. J., Jr., and Bowers, R. F., *Arch. Surg.*, **77**, 837 (1958)

LITERATURE CITED

1. Allen, J. G., *The Physiology and Treatment of Peptic Ulcer* (University of Chicago Press, Chicago, 111, 1959)
2. Code, C. F., *Am. J. Digest. Diseases*, 5, 288 (1960)
3. Dragstedt, L. R., Woodward, E. R., Oberhelman, H. A., Jr., Storer, E. H., and Smith, C. A., *Am. J. Physiol.*, 165, 386 (1951)
4. Heidenhain, R., *Arch. ges. Physiol.*, 19, 148 (1879)
5. Pavlov, I. P., *The Work of the Digestive Glands* (Thompson, W. H., Transl., Chas. Griffith & Co , Ltd., London, Engl., 1902)
6. Dragstedt, L. R., Woodward, E. R., Neal, W. B., Jr., Harper, P. V., and Storer, E. H., *Arch. Surg*, 60, 1 (1950)
7. Gregory, R. A., *J. Physiol.*, 144, 123 (1958)
8. Dragstedt, L. R., Woodward, E. R., Storer, E. H., Oberhelman, H. A., Jr., and Smith, C. A., *Ann. Surg.*, 132, 626 (1950)
9. Farmer, D. A., Howe, C. W., Porell, W. J., and Smithwick, R. H., *Ann. Surg.*, 134, 319 (1951)
10. Ellison, E. H., *Arch. Surg.*, 80, 753 (1960)
11. Noring, O., *Gastroenterology*, 19, 118 (1951)
12. Waddell, W. R., *Ann. Surg*, 143, 520 (1956)
13. Velender, F., *Acta Chir. Scand.*, 101, 49 (1951)
14. Smiddy, F. G., *Gastroenterology*, 32, 1066 (1957)
15. Edwards, L. W., and Herrington, J. L., *Surgery*, 41, 346 (1957)
16. Waddell, W. R., and Williams, H. W., Jr., *Ann Surg.*, 150, 529 (1959)
17. Hollander, F., *Gastroenterology*, 7, 607 (1946)
18. Oberhelman, H. A., Jr, and Dragstedt, L. R., *Proc. Soc. Exptl. Biol. Med.*, 67, 336 (1948)
19. Gregory, R. A., and Tracy, H. J., *Am. J. Digest Diseases*, 5, 308 (1960)
20. Dragstedt, L. R., Woodward, E. R., and Camp, E. H., *Arch. Surg*, 61, 775 (1950)
21. Harkins, H. N., DeVito, R. V., Nyhus, L. M., Stevenson, J. K., and Jones, T. W., *Arch. Surg.*, 79, 133 (1959)
22. Evans, S. O , Jr., Zubiran, J. M., McCarthy, J. D., Ragins, H., Woodward, E. R., and Dragstedt, L. R., *Am. J. Physiol.*, 174, 219 (1953)
23. Schmitz, E. J., Kanar, E. A., Storer, E. H., Sauvage, L. R., and Harkins, H. N., *Surg. Forum, Proc. Congr. Am. Coll. Surgeons* (1952), 17 (Publ. 1953)
24. Kanar, E. A., Schmitz, E. J., Nyhus, L. M., Moore, H. G., Jr., Sauvage, L. R., Storer, E. H., and Harkins, H. N., *Am. J. Physiol.*, 175, 167 (1953)
25. Nyhus, L. M., Kanar, E. A., Moore, H. G., Jr., Sauvage, L. R., Schmitz, E. J., Storer, E. H., and Harkins, H. N., *Surg. Forum, Proc. Congr. Am. Coll. Surgeons*, 4, 346 (1953)
26. Rasmussen, A. T., and Duncan, D., *Proc. Soc. Exptl. Biol. Med.*, 23, 794 (1926)
27. Brooks, J. R., and Moore, F. D., *New Engl. J. Med.*, 249, 1089 (1953)
28. Knox, W. G., and West, J. P., *Ann. Surg.*, 149, 481 (1959)
29. Grimson, K. S., Rowe, C. R., Jr., and Taylor, H. M., *Ann. Surg.*, 135, 621 (1952)
30. Zollinger, R. M., *Arch. Surg.*, 80, 758 (1960)
31. Colp, R., *J. Am. Med. Assoc.*, 162, 1599 (1956)
32. Herrington, J. L., Jr., *Surgery*, 47, 497 (1960)
33. Palumbo, L. T., Sharpe, W. S., Lulu, D. J., Vespa, R., and Colon-Bonet, J., *Ann. Surg.*, 151, 367 (1960)
34. Zubiran, J. M., Kark, A. E., Montalbetti, A. J., Morel, C. J. L., and Dragstedt, L. R., *Arch. Surg.*, 65, 239 (1952)
35. Harkins, H. N., DeVito, R. V., Nyhus, L. M., Stevenson, J. K., and Jones, T. W., *Arch. Surg.*, 79, 133 (1959)
36. Stevenson, J. K., Jesseph, J. E., Jones, T. W., and Harkins, H. N., *Surg. Forum, Proc. Congr. Am. Coll. Surgeons*, 7, 386 (1957)
37. Woodward, E. R., and Tillmanns, V. C., *Surg. Forum, Proc. Congr. Am. Coll. Surgeons*, 6, 301 (1955)
38. Andersson, S., Elwin, C. E., and Uvnas, B , *Gastroenterology*, 34, 636 (1958)
39. Thal, A. P., Perry, J. F., Jr., and Wangensteen, O. H., *Surgery*, 41, 576 (1957)
40. Waddell, W. R., and Kunz, L. J., *New Engl. J. Med* , 255, 555 (1956)
41. Lawrence, W., Jr., Vanamee, P., Peterson, A., McNeer, G., Levin, S., and Randall, H. T., *Surg. Gynecol. Obstet.*, 110, 601 (1960)

42. Wangensteen, O. H., *Cancer of Esophagus and Stomach* (Am. Cancer Soc., New York, 1951)
43. Krause, U , *Acta Chir. Scand.*, **114**, 341 (1958)
44. Janowitz, H. D., and Hollander, F., *J. Appl. Physiol.*, **4**, 53 (1951)
45. Gray, S. J., Ramsey, C. G., and Reifenstein, R. W., *New Engl. J. Med.*, **251**, 835 (1954)
46. Spiro, H. M., Ryan, A. E , and Jones, C. M , *New Engl J. Med.*, **253**, 261 (1955)
47. Hoar, C. S., Jr., and Browning, J R , *New Engl. J. Med.*, **255**, 153 (1956)
48. Glass, G. B. J., Mersheimer, W. L , and Svigals, C. S., *Arch. Surg.*, **62**, 658 (1951)
49. Mersheimer, W. L., Glass, G. B. J., Speer, F. D., Winfield, J. M , and Boyd, L. J., *Ann. Surg* , **136**, 668 (1952)
50. Edkins, J. S., *Am. J. Physiol.*, **34**, 133 (1906)
51. Woodward, E. R., Bigelow, R. R , and Dragstedt, L. R., *Proc. Soc. Exptl. Biol. Med.*, **68**, 473 (1948)
52. Harrison, R. C., Lakey, W. H , and Hyde, H. A., *Ann. Surg.*, **144**, 441 (1956)
53. Woodward, E. R., Robertson, C. R., Fried, W., and Schapiro, H., *Gastroenterology*, **32**, 868 (1957)
54. DuVal, M. K., Jr., and Price, W. E., *Ann. Surg.*, **152**, 410 (1960)
55. McCorkle, H. J., and Harper, H. A., *Ann. Surg.*, **140**, 467 (1954)
56. Weidner, M. G., Jr , Scott, H. W., Jr., Bond, A. G., and Shull, H. J., *Gastroenterology*, **37**, 188 (1959)
57. Ferguson, L. K., *Surg. Clin. North Am.*, **35**, 1693 (1955)
58. Kiefer, E. D., *Gastroenterology*, **37**, 434 (1959)
59. Roberts, K. E., Randall, H. T., Farr, H. W., Kidwell, A. P., McNeer, G. P., and Pack, G. T., *Ann. Surg.*, **140**, 631 (1954)
60 Amdrup, E., and Jorgensen, J. B., *Acta. Chir. Scand.*, **112**, 294 (1957)
61. Henshaw, D. B., Jorgenson, B. J., Davis, H. A., and Stafford, C. E., *Arch. Surg.*, **74** 686 (1957)
62. Webber, B. M., Bender, M. A., and Moore, G. E., *New Engl. J. Med.*, **256**, 285 (1957)
63 Brooks, J. R., and Moore, F. D., *New Engl. J. Med.*, **260**, 20 (1959)
64. Peddie, G. H., Jordan, G. L., Jr , and DeBakey, M. E., *Ann. Surg.*, **146**, 892 (1957)
65. Smith, W. H., *Lancet*, **II**, 745 (1951)
66. Friesen, S. R., and Rieger, E., *Ann. Surg.*, **151**, 517 (1960)
67. Morris, C. C., Jr., Greenfield, L. J., Jordan, G. L., Jr., Peddie, G. H., Gordon, J. R., and DeBakey, M. E., *Ann. Surg.*, **150**, 90 (1959)
68 Machella, T. E., *Ann. Surg.*, **130**, 145 (1949)
69. Mix, C. L., *Surg. Clin. North Am.*, **2**, 617 (1922)
70. Abbott, W. E., Krieger, H., Levey, S., and Bradshaw, J., *Gastroenterology*, **39**, 12 (1960)
71. Liljedahl, S. O., Mattsson, O., Pernow, B., and Wallensten, S., *Acta Chir. Scand.*, **117**, 206 (1959)
72 Henshaw, D. B., Joergenson, E. J., Stafford, C. E., *Arch. Surg.*, **80**, 738 (1960)
73. Everson, T C., *Surgery*, **36**, 525 (1954)
74. Everson, T. C., *Surgery*, **42**, 12 (1957)
75. French, A B., Pollard, H. M , and Ratner, J. T., *Incidence of Postgastrectomy Malabsorption* (Am. Gastroenterological Assoc., April, 1960; not yet published)
76. Ellison, E. M., *Surgery*, **36**, 536 (1954)
77. Everson, T. C., *Intern. Abstr. Surg.*, **95**, 209 (1952)
78 Hayes, M. A., *Surgery*, **37**, 785 (1955)
79. Waddell, W. R., and Wang, C. C., *Ann. N. Y. Acad. Sci.*, **56**, 83 (1952)
80. von Hoesslin, H., *Arch. Hyg.*, **88**, 147 (1919)
81. Beattie, J., Herbert, P. H., and Bell, D. J., *Brit. J Nutrition*, **1**, 202 (1948)
82. Althausen, T. L., Uyeyama, K., and Loran, M. R., *Gastroenterology*, **38**, 942 (1960)
83. Sensenig, D. M., and Campbell, R. E., *Ann. Surg.*, **145**, 81 (1957)
84. Melick, R. A., and Benson, J. A., Jr., *New Engl. J. Med.*, **260**, 976 (1959)
85. Bussabarger, R. A., Freeman, S., and Ivy, A. C., *Am. J. Physiol.*, **121**, 137 (1938)
86. Baird, I. M., and Oleesky, S., *Gastroenterology*, **33**, 284 (1957)
87. Zollinger, R. M., and Ellison, E. H., *J. Am. Med. Assoc.*, **154**, 811 (1954)
88. Thompson, J. A., Bonta, J. A., and Clatworthy, H. W., *Surg. Forum, Proc. Congr. Am. Coll. Surgeons*, **6**, 297 (1955)
89. Green, T. H., Jr., and Hendren, W. H., III, *New Engl. J. Med* , **262**, 118 (1960)
90. Zollinger, R. M., *Arch. Surg.*, **80**, 750 (1960)
91. McCaughan, J. J., Jr., and Bowers, R. F., *Arch. Surg* , **77**, 837 (1958)

92. McLean, L. D., *New Engl J Med.*, 257, 262 (1957)
93. McLean, L. D., *Intern. Abstr. Surg.*, 106, 415 (1958)
94. MacDonald, R. M, Inglefinger, F. J., and Belding, H W., *New Engl J. Med.*, 237, 887 (1947)
95. Halsted, J. A, Gasster, M., and Drenick, E. J, *New Engl. J. Med*, 251, 161 (1954)
96. Mauri-Paolini, A., and Mernoni, E., *Chirugia*, 10, 331 (1955)
97. Birnbaum, D, Rachmilewitz, M., and Grossowicz, N., *Am. J. Digest. Diseases*, 4, 419 (1960)
98 Lyngar, E, *Acta Med. Scand*, Suppl. 138 (1950)
99. Steinberg, M. E., *Surg. Gynecol. Obstet*, 84, 1029 (1947)
100 Schindler, R., and DaGradi, M. E., *Surg. Gynecol. Obstet*, 100, 78 (1955)
101. Benedict, E. B (Personal communication)
102 Jordan, G. L., *Surgery*, 38, 1027 (1955)
103. Goldstein, F., Wirts, C. W, and Kramer, S. *The Relationship of Afferent Limb Stasis and Bacterial Flora to the Production of Postgastrectomy Steatorrhea* (Am. Gastroenterological Assoc, April, 1960; not yet published)
104. Thistlethwaite, J. R, *Surg Gynecol. Obstet.*, 93, 616 (1951)
105. Pfeffer, R. B, Stephenson, H. E, Jr., and Hinton, J. W., *Ann. Surg*, 136, 585 (1952)
106. Martsevich, M. S, *Acad Med. Sci U.S.S R*, 47, 418 (1959)
107. Walker, R S, Watson, C, and Watt, J. K., *Brit Med. J.*, 11, 1438 (1958)
108 Matthews, W. B., and Dragstedt, L. R., *Surg. Gynecol. Obstet.*, 55, 265 (1932)
109. Merendino, K. A., Lannin, B. G., Kolouch, F., Baronofsky, I., Litow, S. S., and Wangensteen, O. H., *Proc. Soc. Exptl. Biol. Med.*, 58, 226 (1945)
110. Kiriluk, L. B., and Merendino, K. A., *Surgery*, 35, 547 (1954)
111. Dillard, D. H, and Merendino, K. A., *Surg. Gynecol. Obstet*, 103, 289 (1956)
112 Farmer, D. A., and Smithwick, R. H., *Surgery*, 35, 557 (1954)
113. Robertson, H. R, *Surg Gynecol. Obstet*, 101, 636 (1955)
114. Moore, F. D., Peete, W. P. J, Richardson, J. E., Erskine, J. M., Brooks, J. R., and Rogers, H, *Ann. Surg*, 132, 652 (1950)
115. Wallensten, S, *Surgery*, 41, 341 (1957)
116. Herrington, J. L., Jr., Edwards, L. W., Classen, K. L, Carlson, R. I, Edwards, W H., and Scott, H. W., Jr., *Ann Surg.*, 150, 499 (1959)
117. Johnson, G, Jr, Sleisenger, H. M., and Beal, J. M., *Ann Surg*, 146, 970 (1957)
118. Dragstedt, L R, Camp, E. H, and Fritz, J. M., *Ann Surg.*, 130, 843 (1949)
119. Grimson, K S, *Ann. Surg*, 150, 513 (1959)
120. Farris, J. M, *Ann. Surg*, 150, 515 (1959)
121. Wangensteen, O. H., *Current Surgical Management*, II, 25 (W. B. Saunders Co, Philadelphia, Pa, 1960)
122. Ferguson, J., Billings, H., Swensen, D, and Hoover, G, *Surgery*, 47, 548 (1960)
123. Cannon, W. B, *Boston Med. Surg. J.*, 161, 720 (1909)
124 Joslin, E. P., and Goodall, H. W., *Boston Med. Surg J.*, 156, 506 (1907)

MALABSORPTION: CELIAC SPRUE[1]

By C. E. Rubin, M. D.[2,3]

Division of Gastroenterology, Department of Medicine
University of Washington, Seattle, Washington

This is a critical review of recent observations on the pathology and pathogenesis of celiac disease in children and idiopathic sprue in adults, both of which are probably the same illness (1, 2, 3). This disease goes by many other names (idiopathic steatorrhea, non-tropical sprue, adult celiac disease, gluten-induced enteropathy, primary intestinal malabsorption, etc.). Until an exact etiologic name is justified we will refer to the disease as celiac sprue.

There have been two major contributions to the understanding of celiac sprue in recent years: (*a*) the recognition of the characteristic intestinal mucosal abnormality (4), and (*b*) the astute observation by Dicke (5) of the therapeutic efficacy of removing dietary wheat.

INTESTINAL LESION IN CELIAC SPRUE

Instruments

Notions regarding intestinal pathology derived from autolyzed post-mortem material or occasional surgical biopsies have been rather confused. More accurate delineation of intestinal histology is now possible because peroral suction biopsies (7 to 17) free of autolysis are easily obtained even in ambulatory patients.

The diagnosis of celiac sprue is best established by biopsy of the distal duodenum or proximal jejunum (10) in patients who have been eating a normal diet containing wheat gluten (18, 19). The proximal three-quarters of the duodenum are best avoided because of difficulties in interpreting mild abnormalities in this area (10) (Figs. 1, 6B).

The ideal instrument for proximal intestinal biopsy should permit several specimens to be taken quickly, safely, and dependably with equal facility in infants and adults. To accomplish this the instrument should be of small diameter, with sufficient flexibility to negotiate sharp turns, but with suffi-

[1] The survey of the literature pertaining to this review was concluded in September, 1960.

[2] The author wishes to acknowledge that the research originating in the University of Washington Gastrointestinal Laboratories is not his alone but rather the result of collaborative effort with his colleagues, Dr. Lloyd L. Brandborg, Dr. Arnold L. Flick, Dr. Walter MacDonald, Mrs. Cherill Murray Parmentier, Miss Patricia Phelps, Mrs. Sharon M. Sribhibhadh, and Mr. Hawley C. Taylor, Jr. He also gratefully acknowledges his debt to his loyal and capable secretary, Mrs. Theresa N. Helton.

[3] The unpublished investigations from the University of Washington Gastrointestinal Laboratory alluded to in this chapter were supported by a research grant C-4320(C2S1) from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

FIG. 1.—Normal small bowel biopsy taken from the junction of the second and third portions of the duodenum in a 13-year-old boy. 75×.

cient rigidity to facilitate rapid advancement. Precise control of suction and a sharp, durable cutting system should insure clean excision of every biopsy with minimal trauma to the specimen and patient.

There are several tubes (11, 12, 15) which theoretically meet these requirements, but only three (8, 9, 10) have had extensive clinical trial. Although the Shiner instrument (8) has been used successfully in several excellent studies, its stiffness and large metal head limit its use in children and make it difficult to pass in adults. The Crosby capsule (9) contains a knife which is activated by suction, permitting sampling of distal intestinal areas not accessible to pull-wire systems. Unfortunately, intubation is time-consuming and the single specimen obtained is often shallow and traumatized. The "multipurpose" tube (10), with a new two-holed capsule, has been used by us in over 200 patients of all ages to obtain over 500 biopsies without complications. With experience, two biopsies may be obtained in 10 to 15 min in over 90 per cent of attempts.

For investigative or special clinical purposes, a hydraulic biopsy tube (16) has been developed that will take numerous biopsies from all levels of the gastrointestinal tract; it delivers each specimen externally within seconds of excision while the tube itself remains *in situ*. Over 200 biopsies have been taken from a dozen patients with this tool. Baker has recently reported good results with a similar hydraulic instrument (17).

Celiac Sprue Histology

The severe celiac sprue lesion may be easily recognized by examining the surface of the fresh biopsy with a hand lens (3); the slender, normal villi offer a marked contrast to the bald or knobby appearance in celiac sprue. Less

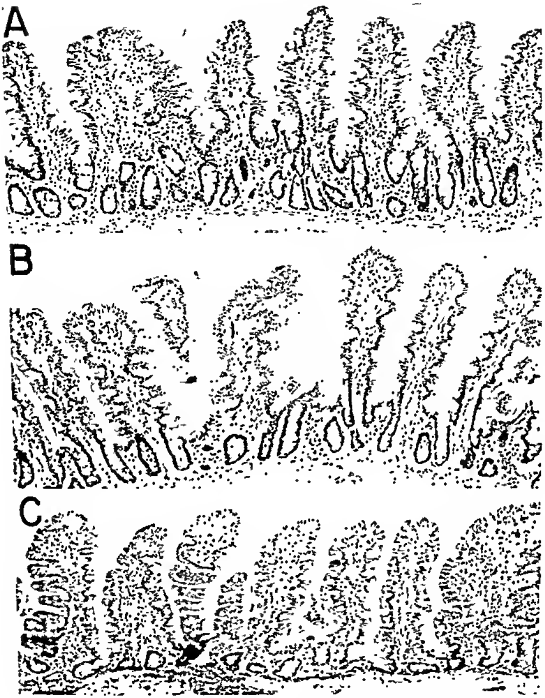


FIG. 2.—Normal proximal jejunal suction biopsies. A: 7 years old, B: 18 years old, C: 50 years old. 75×.

severe lesions may be more difficult to differentiate grossly. At higher magnifications the normal fresh specimen shows an extensive capillary network lying in apposition to the surface columnar cells; this contrasts dramatically with the sparse capillaries of irregular distribution and uneven caliber in celiac sprue (3).

The histology of the intestinal lesion in celiac sprue has been the subject

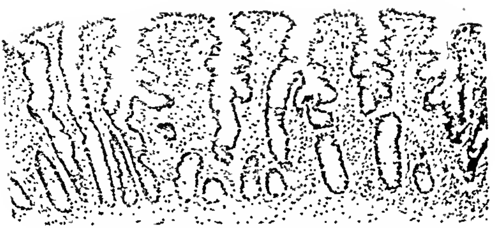


FIG. 3.—Mild proximal jejunal celiac sprue lesion. 75×.

of many papers (3, 4, 19, 20 to 27, 28 to 31, 32, 37). During the past four years over 150 proximal intestinal biopsies from 60 celiac sprue patients of all ages have been carefully evaluated in this laboratory. Over 350 specimens from 150 patients with and without gastrointestinal disease have served as controls. There is considerable variation within the normal range of proximal jejunal biopsy (Figs. 2A: 7 years old; 2B: 18 years old; 2C: 50 years old). Lesions vary in appearance from mild widening of the villi (Fig. 3) to complete loss of villous structure (Fig. 4). Epithelial surface cell changes and infiltration of the lamina propria were usually obvious in the more severe lesions (Fig. 4), although these changes are not invariably present and, in fact, are usually absent in mild lesions (Fig. 3). Our studies would indicate that the most dependable single objective histologic criterion for diagnosing this illness is reduction in epithelial surface (3). Without clinical foreknowledge, independent histologic evaluation by three experienced observers and objective quantitation of epithelial length by a fourth, agreed with the proved clinical diagnoses of celiac sprue in every instance; the lesion was not diagnosed in any of the controls (3).

FIG. 4.—Severe proximal jejunal celiac sprue lesion. 75×.

Padykula *et al.* (32), after counting the increased number of mitoses which are obvious histologically in the severe celiac sprue lesion, found the mitotic index to be twice normal. This finding, as well as the lengthening of the crypts (3, 31, 32, 37), was taken by them to indicate an increase in the rate of cell renewal and cell loss in celiac sprue (32). As they themselves stated, this interpretation depends upon the assumption that the mitotic duration is the same as that seen normally. The duration of mitosis in human intestinal epithelium is unknown either in normal subjects or in those with celiac sprue.

Celiac Sprue Histochemistry

The same six electrophoretically distinct, hydrolytic enzymes were found in the intestinal mucosa of celiac sprue and controls (33). Padykula and her associates (32) have produced a thorough and thoughtful investigation of the morphological and histochemical features of the severe celiac sprue lesion. Histochemically, the germinative crypts of Lieberkuhn generally showed a deficiency of enzymes in both the lesion and in the controls except for strong adenosinetriphophatase activity in normal crypts (32). The complete absence of alkaline phosphatase in both normal and abnormal crypts served as an excellent demarcation between crypt and villous epithelium. The epithelium over the whole length of the normal villus was rich in enzymes (32). In their severely abnormal biopsies, the villous epithelium covered only a short distance, between the end of the lengthened crypt and the surface; it contained normal enzymes although some were reduced in amount. Except for alkaline phosphatase, their severely abnormal surface epithelium was deficient in all enzymes that are normally present at the villus tip and that are presumably needed for transport of nutrients (32). These interesting qualitative, histochemical interpretations will require confirmation by quantitative techniques when they are developed.

Celiac Sprue Electron Microscopy

There have been several preliminary investigations of the intestinal absorptive cell's ultrastructure in celiac sprue in recent years. The electron microscope is admittedly a powerful tool permitting far greater magnification and resolution of structure than the light microscope; but with these advantages come far more complex problems of orientation, sampling, and artifact. The time consumed by careful electron microscopy is incredible; it may take many months to study a single biopsy that could be thoroughly examined by light microscopy in a few minutes. The early work of the Hartmans and their colleagues (34) in this field utilized a biopsy before and after a gluten-free diet in a patient with celiac sprue. Despite the tissue damage resulting from delay in fixation and other technical problems, they demonstrated decreased size and number of the microvilli in the brush border in celiac sprue. In a preliminary note, Zetterqvist & Hendrix (35) illustrated apparent improvement in the microvilli of a single case of celiac sprue after institution of a

FIG. 5.—Electron micrograph of a proximal jejunal biopsy of mild celiac sprue lesion. (*fixation-collidine buffered osmic acid, embedding-epoxy resin*). A. Upper halves of several absorptive cells facing the intestinal lumen including their brush borders MV = microvilli at the brush border; TW = terminal web area beneath brush border. 9500×. B. Higher magnification of the area in Figure 9A within the black rectangle. D = desmosome, i.e., area of attachment between cells; M = mitochondrion; ER = endoplasmic reticulum. 33400×.

gluten-free diet. The electron micrographs from 13 celiac sprue patients of biopsies taken by Padykula *et al.* (32) confirm unequivocally that the microvilli of the surface cells are reduced in size and number. They described other changes in the abnormal surface cells: mitochondrial enlargement, decreased mitochondrial density, and decreased membranous components of the endoplasmic reticulum (32). Observations by Phelps (36) confirm the changes in the microvilli and point up some of the difficulties in sampling and orientation. For example, normal microvilli cover absorptive epithelium in

the mouth of the crypts only a few cells distant from the abnormal microvilli of the surface cells. Exact orientation is imperative because the most severe abnormalities and often the only ones are seen on the well-oriented surface facing the lumen. This requires great skill in trimming the block and repeated examination of thick sections by phase microscopy. Phelps has noted a wide spectrum of change in untreated celiac sprue; the milder lesions show less microvillous alteration in the surface cells and no evident changes in mitochondria or membranous components (Figs. 5A and 5B).

Interpretive Difficulties

The greatest single source of error is the artifact caused by tangential sectioning. Figure 6A illustrates a section at the edge of a biopsy from a 10-month-old boy exhibiting a tangential artifact which simulates a moderately severe celiac sprue lesion; Figure 6B reveals normal duodenal villi in serial sections taken further along in the same block within its well-oriented central core. The careful orientation of tissue and the precise sectioning technique required to minimize this source of misinterpretation have been

Fig. 6.—Serial sections from the edge and center of a normal mid-duodenal biopsy in a ten-month-old boy. A. Tangential artifact at the edge of the section. B. Normal duodenum in the well-oriented area of the same block.

FIG. 7.—Normal mid-duodenal suction biopsy showing the Brunner artifact.

described in detail elsewhere (3, 10). Tangential artifact is inevitable even in perfectly oriented specimens because some villi are always askew at the time of fixation; for this reason we use only the very best oriented areas for diagnostic interpretation and consider the presence of four or more completely normal villi in a row sufficient to exclude the diagnosis of celiac sprue (Figs. 2A, 2B, 2C). Normal Brunner's glands may replace the mucosa and flatten the villi in the first and second portions of the duodenum (Fig. 7); lymphoid nodules, especially in children, cause interpretative difficulties (3, 10). Both of these normal variants may lead to incorrect diagnoses of celiac sprue.

The lesion is a diffuse one (3, 37) which decreases in severity caudally (3, 18, 37). Sampling error is not a great problem if the level of biopsy is controlled. If ten biopsies are taken near the duodenojejunal junction on the same day in the same celiac sprue patient, there will be only mild variation in their histologic severity and all will be diagnostic (3).

DIAGNOSTIC SPECIFICITY

There are claims that the celiac sprue lesion may be seen after gastrectomy (38, 39), neomycin treatment (40), in severe malnutrition (41), with Asiatic cholera (42), and in non-specific diarrheas (43). We have examined biopsies from all of these entities in our own patients or in tissue kindly loaned us by other investigators; as a result we believe that the great majority of these biopsies are normal. Many of the illustrations in the papers cited are within the normal range and others represent understandable misinterpretations of tangential artifacts. Occasionally, Brunner or lymphoid artifacts are represented as the celiac sprue lesion. Those few biopsies which

were clearly abnormal in these conditions may well come from patients with tropical or celiac sprue.

In this laboratory, proximal jejunal biopsies taken from patients having the following diseases with associated steatorrhea were within normal limits: chronic pancreatitis, cystic fibrosis of the pancreas, agammaglobulinemia, Zollinger-Ellison syndrome, pernicious anemia, partial gastrectomy, partial intestinal resection, and jejunal diverticulosis. Other conditions which might theoretically be associated with intestinal alterations proved to have normal proximal jejunal biopsies: severe malnutrition, iron deficiency, myxedema, acromegaly, hypopituitarism, scleroderma, infectious diarrhea, and selective vitamin B_{12} malabsorption with proteinuria (106).

Clinically, tropical sprue is different from celiac sprue in its lack of response to a gluten-free diet (44 to 47) and in its tendency to spontaneous remission (48, 49). Its pathogenesis is unknown. Histologically, we have been unable to discern obvious differences between these two entities although those with greater experience with the tropical variety believe they can (50). Thus, with the possible exception of tropical sprue, the lesion in celiac sprue seems, in our present state of knowledge, to be specific and diagnostic (3). Should the morphologic appearance prove less specific with further experience, the ability of wheat to cause the lesion would still be pathognomonic (See GLUTEN IN CELIAC SPRUE, HISTOLOGIC OBSERVATIONS).

There are a few other diseases that may be diagnosed by the unique appearance of their proximal intestinal mucosae: In Whipple's disease (23, 37, 51, 52, 53), the villi are stuffed with macrophages containing sickle-shaped particles (54) which are positive with the *p*-aminosalicylic acid (PAS) stain.

Acanthocytosis (55 to 59) is a rare but interesting hereditary disorder, characterized by steatorrhea, spiny red cells, neurologic abnormalities, and absence of *beta*-lipoprotein (59). Characteristically (59), intestinal absorptive cells covering the upper portion of the villi are packed with cytoplasmic vacuoles; their brush borders are usually intact; the crypt cells and the rest of the villous epithelium are uninvolved (Fig. 8A—normal villus; Fig. 8B—acanthocytosis). Histologically, one would guess that the intestinal absorptive cells have failed to metabolize lipid material that was absorbed from the intestinal lumen, although the histochemistry of the vacuoles remains to be established.

The finding of Giardia lamblia on the external surface of normal villi suggests the diagnosis of giardiasis (60), a cause of steatorrhea which responds to atabrine. These organisms take the H and E stain poorly and careful search under oil is needed to find them.

Protein-losing gastroenteropathy (61 to 64) is best demonstrated by the polyvinyl pyrrolidone (PVP) test (65). It may be caused by various known exudative lesions such as giant hypertrophic gastritis (66), gastric cancer (67), regional enteritis (68, 69), ulcerative colitis (68, 69), sarcoid (70), periarteritis (70), non-specific granuloma (70), and in rare cases of heart

failure (69). There is a remaining idiopathic group in which no intestinal mucosal lesion is apparent; recently, dilated lymphatic vessels have been observed in small bowel biopsies from such cases (69) and one wonders whether some unsuspected obstructive process may cause this lymphectasia and be the source of protein leakage.

Regional enteritis is theoretically diagnosable by suction biopsy, but, from a practical point of view, it is a difficult diagnosis to make because the

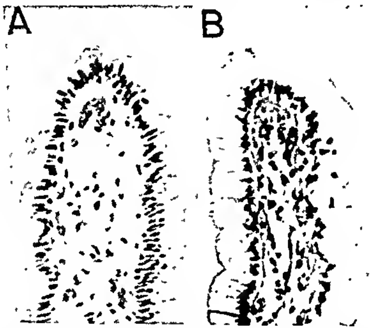


FIG. 8.—Tips of well-oriented villi. 300×. A. Normal B. Acanthocytosis

lesion is patchy and usually distally located. It is difficult to approach because impaired peristalsis may keep the tube from reaching the area of mucosal involvement (60); furthermore, the most characteristic changes are beyond the depth of suction biopsy.

GLUTEN IN CELIAC SPRUE

In 1950, Dicke (5) demonstrated that removal of wheat from the diet caused the symptoms and signs of celiac disease to disappear, whereas reintroduction of the offending agent caused recurrence. In a series of classical experiments Dicke (6), Weijers & van de Kamer (6, 71, 72, 73) confirmed and extended these observations. The Dutch workers recommended that wheat, rye, barley, and oats be removed from the diet (72) although oats in England now seem relatively harmless (74). The efficacy of a meticulous gluten-free diet in celiac sprue was quickly confirmed by other observers in both children (75, 76) and in adults (45, 77 to 80).

Biochemical Observations

It was soon demonstrated that the injurious fraction of wheat was contained in gluten, its water-insoluble fraction (6). Gluten extracted with 50 per cent ethanol yields gliadin, an unusual protein containing 43 per cent of glutamine (72). Gliadin by mouth is just as toxic as gluten in celiac sprue (72), but glutamine or glutamic acid alone are without effect (81). After digestion with pepsin or trypsin, gliadin yields a six-or seven-amino acid polypeptide (82) which has not lost its toxicity (82). Furthermore, destruction of all of the sulphur-bridge interchain bonds in this polypeptide also does not destroy the toxicity (83). On the other hand, if gliadin is digested with papain (82) or an extract of fresh porcine small intestinal mucosa (84), it is split up into its amino acids prominent among which are glutamine and proline (82); this digest has lost its ability to exacerbate celiac sprue (82). It is of interest that although tryptic or peptic digestion fails to release proline from gliadin, it does release it from zein (corn protein) and casein (milk protein), both of which are non-toxic in celiac sprue (82). If gliadin is deamidated by hydrolysis with weak acid, it loses its toxic effect in celiac sprue (81); this treatment converts the glutaminal residues in the gliadin to the free glutamyl residues without having much effect on the peptide linkages.

Some observers have reported that an oral dose of gliadin in celiac sprue causes a greater rise in the blood levels of glutamine or glutamine-containing polypeptides (85) than it does in normal subjects (86 to 90), although this is by no means a uniform (87, 88) or a specific phenomenon (88, 90). An unusual fast-moving peptide containing no proline has been observed in the serum of juvenile celiac sprue patients on a gluten-containing diet (91). The exact chemical nature of the substances measured in the blood by the various methods employed is uncertain. Excessive levels of several substances in the urine have been observed in celiac sprue patients on a gluten-containing diet: *p*-hydroxyphenylacetic acid (92), 5-hydroxyindoleacetic acid (93), indole-3-acetic acid (94), glutamate (95), and indican (96).

On the basis of these fragmentary biochemical observations, various theories regarding the pathogenesis of celiac sprue have been proposed. Central to most of them is the hypothesis that celiac sprue is an inborn error of metabolism with a deficiency of some essential enzyme necessary for metabolizing gliadin and related proteins. It is not a deficiency of leucylaminopeptidase (97). There are several other speculations regarding the pathogenesis of this disease that have less evidence to support them.

Steatorrhea is the hallmark of all malabsorption and celiac sprue is no exception. It is a fascinating fact that patients with this disease absorb unsaturated fats more efficiently than saturated fats (71, 98). They may indeed have a normal coefficient of fat absorption and improve clinically if all of the fat in their diet is unsaturated (98). A considerable elevation in most of the serum lipid components occurred in a child with celiac disease when the source of dietary fat was shifted from corn oil to cream despite the

resultant decrease in fat absorption (99). An understanding of this mechanism might conceivably have wide-spread therapeutic applications.

Histologic Observations

The nature of the relationship between gluten and the intestinal lesion is obscure Although the lesion may improve (28) and even disappear (19) in young patients after gluten removal, it persists in the great majority of adults and has been observed even after five years of clinical remission on a careful gluten-free diet (28). Furthermore, there is a puzzling lack of correlation between the histologic severity of the proximal lesion and the patient's clinical status (19, 28, 37).

Recent observations may help to explain these apparent inconsistencies and offer some insight into the pathogenesis of celiac sprue. Using the hydraulic biopsy tube (16), observers saw no normal areas in samples obtained from the entire length of the small intestine of two adults with severe celiac sprue whose diet contained gluten; however, the lesion was definitely less severe distally (18, 60). In marked contrast, two well patients on a gluten-free diet had varying lengths of normal appearing and normal functioning distal jejunum and ileum (3,100). These observations suggest that the proximal bowel is most severely damaged because it is exposed to the highest concentration of undigested dietary gluten. With institution of a gluten-free diet the intestinal abnormality may recede in a proximal direction, the milder, more distal lesions recovering first and the more severe proximal ones last, or not at all. Irreversible proximal damage may result from years of gluten exposure.

The length of diseased proximal bowel may correlate better with the clinical severity of each case of celiac sprue than does the appearance of a random proximal biopsy. This theory gains support from the findings in two adults with this disease who were on meticulous gluten-free diets; the patient whose fat absorption became normal had a far shorter length of abnormal proximal intestine than the other patient who had residual steatorrhea (100). Obviously, studies which rely on proximal biopsy alone yield an incomplete picture because examination of one end of the intestine does not permit conclusions regarding all of it Biopsies taken only from the duodenum and proximal jejunum do not assess completely the effect of gluten removal; the most severely involved proximal segment may change slowly or be irreversibly damaged while unknown lengths of the less exposed distal segment may have recovered. Furthermore, it is essential to take biopsies from exactly the same level if any comparison is to be made before and after treatment. The reason for this is now clear. when one takes a group of proximal intestinal biopsies on the same day, those which are even a few inches distal may appear less diseased (100).

The normal appearing distal intestine in treated celiac sprue (3) offers a unique opportunity for determining the relationship between gluten and the mucosal lesion. Wheat was instilled thrice daily via an inlying tube into

the normal appearing bowel of two patients with idiopathic sprue while their gluten-free diets were continued (100). In both patients this procedure not only caused a severe exacerbation of clinical sprue but also changed the appearance of the previously normal ileal mucosa to that characteristically seen in celiac sprue proximally (100). Widespread ileal damage caused malabsorption of vitamin B_{12} for many months in one of these patients. Similar wheat instillation in normal controls produced neither symptoms nor histologic changes. Unpublished experiments performed in this laboratory prove that 50 gm. of wheat can severely damage the normal ileal mucosa in celiac sprue within 6 hr. of its instillation and, furthermore, that the morphologic alterations can disappear entirely within 30 hours!

With a meticulous gluten-free diet the proximal intestinal lesion in juvenile celiac sprue may improve or disappear completely within 3 months to a year (19); in one young patient histologic improvement has been observed in as little as three weeks (60). In adults, some proximal mucosal restitution is frequently revealed by serial biopsy during a compulsive gluten-free diet (60, 101); the changes however are far less dramatic than those seen in children. Figure 9A illustrates a severe proximal lesion in a 35-year-old woman with recent onset of celiac sprue; Figure 9B illustrates the

FIG. 9.—Biopsies at the duodenojejunal junction in a 35-year-old patient with celiac sprue. A. Before treatment. B. After six months of a gluten-free diet.

resultant decrease in fat absorption (99). An understanding of this mechanism might conceivably have wide-spread therapeutic applications.

Histologic Observations

The nature of the relationship between gluten and the intestinal lesion is obscure. Although the lesion may improve (28) and even disappear (19) in young patients after gluten removal, it persists in the great majority of adults and has been observed even after five years of clinical remission on a careful gluten-free diet (28). Furthermore, there is a puzzling lack of correlation between the histologic severity of the proximal lesion and the patient's clinical status (19, 28, 37).

Recent observations may help to explain these apparent inconsistencies and offer some insight into the pathogenesis of celiac sprue. Using the hydraulic biopsy tube (16), observers saw no normal areas in samples obtained from the entire length of the small intestine of two adults with severe celiac sprue whose diet contained gluten; however, the lesion was definitely less severe distally (18, 60). In marked contrast, two well patients on a gluten-free diet had varying lengths of normal appearing and normal functioning distal jejunum and ileum (3,100). These observations suggest that the proximal bowel is most severely damaged because it is exposed to the highest concentration of undigested dietary gluten. With institution of a gluten-free diet the intestinal abnormality may recede in a proximal direction, the milder, more distal lesions recovering first and the more severe proximal ones last, or not at all. Irreversible proximal damage may result from years of gluten exposure.

The length of diseased proximal bowel may correlate better with the clinical severity of each case of celiac sprue than does the appearance of a random proximal biopsy. This theory gains support from the findings in two adults with this disease who were on meticulous gluten-free diets; the patient whose fat absorption became normal had a far shorter length of abnormal proximal intestine than the other patient who had residual steatorrhea (100). Obviously, studies which rely on proximal biopsy alone yield an incomplete picture because examination of one end of the intestine does not permit conclusions regarding all of it. Biopsies taken only from the duodenum and proximal jejunum do not assess completely the effect of gluten removal; the most severely involved proximal segment may change slowly or be irreversibly damaged while unknown lengths of the less exposed distal segment may have recovered. Furthermore, it is essential to take biopsies from exactly the same level if any comparison is to be made before and after treatment. The reason for this is now clear: when one takes a group of proximal intestinal biopsies on the same day, those which are even a few inches distal may appear less diseased (100).

The normal appearing distal intestine in treated celiac sprue (3) offers a unique opportunity for determining the relationship between gluten and the mucosal lesion. Wheat was instilled thrice daily via an inlying tube into

the normal appearing bowel of two patients with idiopathic sprue while their gluten-free diets were continued (100). In both patients this procedure not only caused a severe exacerbation of clinical sprue but also changed the appearance of the previously normal ileal mucosa to that characteristically seen in celiac sprue proximally (100). Widespread ileal damage caused malabsorption of vitamin B_{12} for many months in one of these patients. Similar wheat instillation in normal controls produced neither symptoms nor histologic changes. Unpublished experiments performed in this laboratory prove that 50 gm. of wheat can severely damage the normal ileal mucosa in celiac sprue within 6 hr. of its instillation and, furthermore, that the morphologic alterations can disappear entirely within 30 hours!

With a meticulous gluten-free diet the proximal intestinal lesion in juvenile celiac sprue may improve or disappear completely within 3 months to a year (19); in one young patient histologic improvement has been observed in as little as three weeks (60). In adults, some proximal mucosal restitution is frequently revealed by serial biopsy during a compulsive gluten-free diet (60, 101); the changes however are far less dramatic than those seen in children. Figure 9A illustrates a severe proximal lesion in a 35-year-old woman with recent onset of celiac sprue; Figure 9B illustrates the

FIG. 9.—Biopsies at the duodenojejunal junction in a 35-year-old patient with celiac sprue. A. Before treatment. B. After six months of a gluten-free diet.

resultant decrease in fat absorption (99). An understanding of this mechanism might conceivably have wide-spread therapeutic applications.

Histologic Observations

The nature of the relationship between gluten and the intestinal lesion is obscure. Although the lesion may improve (28) and even disappear (19) in young patients after gluten removal, it persists in the great majority of adults and has been observed even after five years of clinical remission on a careful gluten-free diet (28). Furthermore, there is a puzzling lack of correlation between the histologic severity of the proximal lesion and the patient's clinical status (19, 28, 37).

Recent observations may help to explain these apparent inconsistencies and offer some insight into the pathogenesis of celiac sprue. Using the hydraulic biopsy tube (16), observers saw no normal areas in samples obtained from the entire length of the small intestine of two adults with severe celiac sprue whose diet contained gluten; however, the lesion was definitely less severe distally (18, 60). In marked contrast, two well patients on a gluten-free diet had varying lengths of normal appearing and normal functioning distal jejunum and ileum (3,100). These observations suggest that the proximal bowel is most severely damaged because it is exposed to the highest concentration of undigested dietary gluten. With institution of a gluten-free diet the intestinal abnormality may recede in a proximal direction, the milder, more distal lesions recovering first and the more severe proximal ones last, or not at all. Irreversible proximal damage may result *from years of gluten exposure.*

The length of diseased proximal bowel may correlate better with the clinical severity of each case of celiac sprue than does the appearance of a random proximal biopsy. This theory gains support from the findings in two adults with this disease who were on meticulous gluten-free diets; the patient whose fat absorption became normal had a far shorter length of abnormal proximal intestine than the other patient who had residual steatorrhea (100). Obviously, studies which rely on proximal biopsy alone yield an incomplete picture because examination of one end of the intestine does not permit conclusions regarding all of it. Biopsies taken only from the duodenum and proximal jejunum do not assess completely the effect of gluten removal; the most severely involved proximal segment may change slowly or be irreversibly damaged while unknown lengths of the less exposed distal segment may have recovered. Furthermore, it is essential to take biopsies from exactly the same level if any comparison is to be made before and after treatment. The reason for this is now clear: when one takes a group of proximal intestinal biopsies on the same day, those which are even a few inches distal may appear less diseased (100).

The normal appearing distal intestine in treated celiac sprue (3) offers a unique opportunity for determining the relationship between gluten and the mucosal lesion. Wheat was instilled thrice daily via an indwelling tube into

the normal appearing bowel of two patients with idiopathic sprue while their gluten-free diets were continued (100). In both patients this procedure not only caused a severe exacerbation of clinical sprue but also changed the appearance of the previously normal ileal mucosa to that characteristically seen in celiac sprue proximally (100). Widespread ileal damage caused malabsorption of vitamin B_{12} for many months in one of these patients. Similar wheat instillation in normal controls produced neither symptoms nor histologic changes. Unpublished experiments performed in this laboratory prove that 50 gm. of wheat can severely damage the normal ileal mucosa in celiac sprue within 6 hr. of its instillation and, furthermore, that the morphologic alterations can disappear entirely within 30 hours!

With a meticulous gluten-free diet the proximal intestinal lesion in juvenile celiac sprue may improve or disappear completely within 3 months to a year (19); in one young patient histologic improvement has been observed in as little as three weeks (60). In adults, some proximal mucosal restitution is frequently revealed by serial biopsy during a compulsive gluten-free diet (60, 101); the changes however are far less dramatic than those seen in children. Figure 9A illustrates a severe proximal lesion in a 35-year-old woman with recent onset of celiac sprue; Figure 9B illustrates the

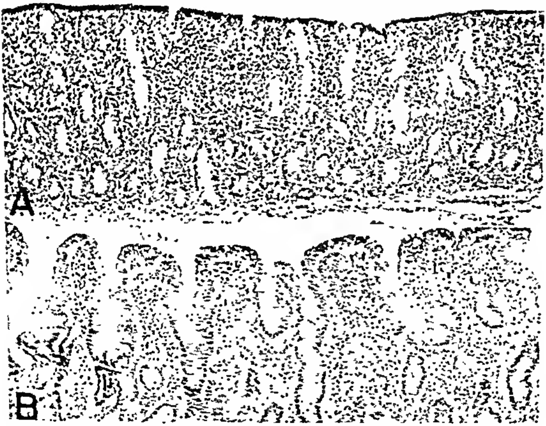


FIG. 9.—Biopsies at the duodenojejunal junction in a 35-year-old patient with celiac sprue. A. Before treatment. B. After six months of a gluten-free diet.

milder proximal pathology after six months of a gluten-free diet. This is one of the most dramatic responses to gluten removal recorded thus far; undoubtedly instances of complete intestinal recovery in adults will be reported

It is thus apparent that exhibition of wheat is followed regularly by intestinal injury in celiac sprue and that its removal is followed by restitution of intestinal integrity. Such changes do not occur in controls. These findings suggest a specific metabolic defect in celiac sprue which precedes injury by wheat Clinical family studies suggest a genetic basis (48, 102 to 105) for the defect and this is supported by the demonstration of characteristic biopsies in two succeeding generations and in identical twins (28). The evidence to date suggests that neither gluten nor the intestinal abnormality is the primary cause of celiac sprue. It appears, instead, that they are intervening links in a causal chain which begins as a specific metabolic defect and ends in a clinically apparent illness. Current vigorous research promises to yield further clues regarding the missing links.

LITERATURE CITED

1. Fairly, H H , *Trans Roy. Soc. Trop. Med Hyg*, 30, 9 (1936)
2. Cooke, W T , *Brit Med J.*, II, 261 (1958)
3. Rubin, C E , Brandborg, L. L , Phelps, P. C , and Taylor, H C., Jr., *Gastroenterology*, 38, 28 (1960)
4. Paulley, J. W , *Brit Med J.*, II, 1318 (1954)
5. Dicke, W. K. *Coeliakie Een onderzoek naar de nadelige invloed van sommige graansoorten op de lijder aan Coeliakie* (Doctoral thesis, Univ. of Utrecht, Netherlands, 1950)
6. Dicke, W K , Weijers, H A., and van de Kamer, J H , *Acta Paediat* , 42, 34 (1953)
7. Royer, M , Croxatto, O , Biempica, L , and Balcazar-Morrison, A J , *Prena méd arg* , 42, 2515 (1955)
8. Shiner, M , *Lancet*, I, 85 (1956)
9. Crosby, W H , and Kugler, H W , *Am J. Digest Diseases*, 2, 236 (1957)
10. Brandborg, L L , Rubin, C E , and Quinton, W. E , *Gastroenterology*, 37, 1 (1959)
11. de Larrechea, I , Schapira, A , and Ramos Mejia, M M , *Prensa méd arg* , 46, 1328 (1959)
12. Henning, N , Zeitler, G , and Neugebauer, L., *Deut. med Wochschr* , 94, 1961 (1959)
13. Shiner, M . *Proc. Roy. Soc Med* , 52, 10 (1959)
14. Carey, J B., Jr , and Pankey, G A., *Gastroenterology*, 38, 961 (1960) (Abstr.)
15. Ross, J R , and Moore, V. S , *Gastroenterology*, 38, 976 (1960) (Abstr.)
16. Flick, A L., Quinton, W E., and Rubin, C E , *Gastroenterology*, 38, 964 (1960) (Abstr.). To be published in detail, *Gastroenterology*, 40, (Jan. 1961)
17. Baker, S J , and Hughes, A., *Lancet*, II, 686 (1960)
18. Rubin, C. E., *Gastroenterology*, 39, 260 (1960)
19. Anderson, C M., *Arch Diseases Childhood* (To be published)
20. Shiner, M , *J. Mt. Sinai Hosp.*, 24, 273 (1957)
21. Sakula, J , and Shiner, M., *Lancet*, II, 876 (1957)
22. Himes, H. W., and Adlersberg, D , *Gastroenterology*, 35, 142 (1958)
23. Bolt, R. J., Pollard, M., and Standaert. L., *New Engl J. Med* , 259, 32 (1958)
24. Kelley, M. L , and Terry, R , *Am. J. Med.*, 25, 460 (1958)
25. Culver, P. J., Benson, J. A., Jr., Strauss, E., and Jones, C. M., *Gastroenterology*, 36, 459 (1959)
26. Paulley, J. W., *Lancet*, II, 646 (1959)
27. de Larrechea, I , Schapira, A , and Ramos Mejia, M. M , *Prensa méd. arg* , 46, 1170 (1959)
28. Rubin, C. E., Brandborg, L. L., Phelps, P. C , Taylor, H. C., Jr., Murray, C. V., Stemler, R ,

Howry, C., and Volwiler, W., *Gastroenterology*, 38, 517 (1960)
29. Fone, D. J., Meynell, M. J., Harris, E. L., Cooke, W. T., Brewer, D. B., and Cox, E. V., *Lancet*, I, 933 (1960)
30. Shiner, M., and Doniach, I., *Gastroenterology*, 38, 419 (1960)
31. Doniach, I., and Shiner, M. V., *Brit. Radiol.*, 33, 238 (1960)
32. Padykula, H. A., Strauss, E. W., Ladman, A. J., and Gardner, F. H. (To be published)
33. Bolt, R. J., Pollard, H. M., and McCool, S., *Am. J. Clin. Pathol.*, 34, 43 (1960)
34. Hartman, R. S., Butterworth, C. E., Jr., Hartman, R. E., Crosby, W. H., and Shirai, A., *Gastroenterology*, 38, 506 (1960)
35. Zetterqvist, H., Hendrix, T. R., *Bull. Johns Hopkins Hosp.*, 106, 240 (1960)
36. Phelps, P. C., and co-workers (University of Washington Gastrointestinal Laboratories. Unpublished data)
37. Thurlbeck, W. M., Benson, J. A., and Dudley, H. R., *Am. J. Clin. Pathol.*, 34, 108 (1960)
38. Joske, R. A., and Blackwell, J. B., *Lancet*, II, 379 (1959)
39. Baird, I. M., and Dodge, O. G., *Quart. J. Med.*, 26, 393 (1957)
40. Jacobson, E. D., Prior, J. T., and Faloon, W. W., *J. Lab. Clin. Med.*, 56, 245 (1960)
41. Zubirán, S., *Am. Coll. Physicians* (Postgraduate Course in Gastroenterology, New Orleans, La., March, 1960)
42. Gangarosa, E. J., Beisel, W. R., Benyajati, C., Sprinz, H., and Piyaratn, P., *Am. J. Trop. Med. Hyg.*, 9, 125 (1960)
43. Yesner, R., Schwartz, R. D., and Spiro, H. M., *Yale J. Biol. Med.*, 32, 361 (1960)
44. Webb, J. F. (M. D. thesis, Duke Univ., Durham, North Carolina, 1956)
45. French, J. M., Hawkins, C. F., and Smith, N., *Quart. J. Med.*, 26, 418 (1957)
46. Baker, S. J., *Indiana J. Med. Sci.*, 11, 687 (1957)
47. Asenjo, C. F., Rodriquez-Molina, R., Cancio, M., and Bernabe, R. A. *Am. J. Trop. Med. Hyg.*, 7, 347 (1958)
48. Davidson, L. S. P., and Fountain, J. R., *Brit. J. Med.*, I, 1157 (1950)
49. Hazari, O. K., and Woodruff, A. W., *Brit. Med. J.*, II, 344 (1958)
50. Butterworth, C. E., Jr., Smith, B. W., and Perez-Santiago, E., *World Congr. Gastroenterol.*, 1, 629 (1958) (Williams & Wilkins Co., Baltimore, Md.)
51. Brodoff, M., Hoffman, W. A., DeLuca, V. A., Jr., and Spiro, H. M., *J. Am. Med. Assoc.*, 171, 154 (1959)
52. Hargrove, M. D., Jr., Verner, J. B., Jr., Patrick, R. L., and Ruffin, J. M., *J. Am. Med. Assoc.*, 173, 1125 (1960)
53. England, M. T., French, J. M., and Rawson, A. B., *Gastroenterology*, 39, 219 (1960)
54. Sieracki, J. A., *Arch. Pathol.*, 66, 464 (1958)
55. Bassen, F. A., and Kornzweig, A. L., *Blood*, 5, 381 (1950)
56. Singer, K., Fisher, B., and Perlstein, M. A., *Blood*, 7, 577 (1952)
57. Kornzweig, A. L., and Bassen, F. A., *Arch. Ophthalmol.*, 59, 818 (1958)
58. Druez, G., *Rev. hématol.*, 14, 3 (1959)
59. Salt, H. B., Wolff, O. H., Lloyd, J. K., Forsbrooke, A. S., Cameron, A. H., and Hubble, D. V., *Lancet*, II, 325 (1960)
60. Rubin, C. E., and co-workers (University of Washington Gastrointestinal Laboratories. Unpublished data)
61. Gordon, R. S., Jr., *Lancet*, I, 325 (1959)
62. Schwartz, M., and Jarnum, S., *Lancet*, I, 327 (1959)
63. Littman, A., *Gastroenterology*, 39, 234 (1960)
64. Editorial, *Lancet*, I, 351 (1959)
65. Gordon, R. S., Jr., Bartter, F. C., and Waldmann, T. *Ann. Internal Med.*, 51, 553 (1959)
66. Citrin, Y., Sterling, K., and Halsted, J. A., *New Engl. J. Med.*, 257, 906 (1957)
67. Jarnum, S., and Schwartz, M., *Gastroenterology*, 38, 769 (1960)
68. Steinfeld, J. L., Davidson, J. D., and Gordon, R. S., Jr., *J. Clin. Invest.*, 36, 931 (1959)
69. Waldmann, T. A., Steinfeld, J. L., Dutcher, T. F., Davidson, J. D., and Gordon, R. S., Jr., *Hypoproteinemia Due To Gastrointestinal Disorders* (Paper presented at the Am. Gastroenterological Assoc. Ann. Meet., New Orleans, La., April, 1960)
70. Holman, H., Nickel, W. F., Jr., and Sleisenger, M. S., *Am. J. Med.*, 27, 963 (1959)
71. Weijers, H. A., and van de Kamer,

J. H., *Acta Paediat.*, 42, 97 (1953)
72. van de Kamer, J. H., Weijers, H. A., and Dicke, W. K., *Acta Paediat.*, 42, 223 (1953)
73. Weijers, H. A., van de Kamer, J. H., and Dicke, W. K., *Advances in Pediat.*, 9, 277 (1957)
74. Moulton, A. L. C., *Arch. Diseases Childhood*, 34, 41 (1960)
75. Anderson, C. M., Frazer, A. C., French, J. M., Gerrard, J. W., Sammons, H. G., and Smellie, J. M., *Lancet*, I, 836 (1952)
76. Sheldon, W., and Lawson, D., *Lancet*, II, 902 (1952)
77. McIver, C., *Lancet*, II, 1112 (1952)
78. Ruffin, J. M., Carter, D. D., Johnston, D. H., and Baylin, G. J., *New Engl. J. Med.*, 250, 281 (1954)
79. Schwartz, M. D., Sleisenger, M. H., Pert, J. H., Roberts, K. E., Randall, H. T., and Almy, T. P., *Gastroenterology*, 32, 232 (1957)
80. Finlay, J. M., and Wightman, K. J. R., *Ann. Internal Med.*, 45, 191 (1956)
81. van de Kamer, J. H., and Weijers, H. A., *Acta Paediat.*, 44, 465 (1955)
82. Krainick, V. H. G., and Mohn, G., *Helv. Paediat. Acta*, 14, 124 (1959)
83. van Roon, J. H., and Haex, A. J. C., *New developments in the analysis of the gluten-effect on patients suffering from idiopathic steatorrhea* (Paper presented at the Intern. Congr. Gastroenterology, Leiden, Netherlands, April, 1960)
84. Frazer, A. C., *Proc. Roy. Soc. Med.*, 49, 1009 (1956)
85. Weijers, H. A., and van de Kamer, J. H., *Acta Paediat.*, 48, 17 (1959)
86. Weijers, H. A., and van de Kamer, J. H., *Acta Paediat.*, 44, 536 (1955)
87. Alvey, C., Anderson, C. M., and Freeman, M., *Arch. Diseases Childhood*, 32, 434 (1957)
88. Payne, W. W., and Jenkinson, V., *Arch. Diseases Childhood*, 33, 413 (1958)
89. Puranen, J., Puranen, A., and Hallman, N., *Ann. Paediat. Fenniae*, 4, 203 (1958)
90. Visakorpi, J. A., *Ann. Paediat. Fenniae*, 5, 67 (1959)
91. Grüttner, R., Mellin, R., and Branstedt, F., *Klin. Wochschr.*, 37, 237 (1959)
92. Boscott, R. J., and Cooke, W. T., *Quart. J. Med.*, 23, 307 (1954)
93. Kowlessar, O. D., Williams, R. C., Law, D. H., and Sleisenger, M. H., *New Engl. J. Med.*, 259, 340 (1958)
94. Haverback, B. J., Dyce, B., and Thomas, H. V., *New Engl. J. Med.*, 262, 754 (1960)
95. Marko, A. M., and Gerrard, J. W. (To be published)
96. Marko, A. M., and Gerrard, J. W. (To be published)
97. van Roon, J. H., and Haex, A. J. C., *Methods & Results of the Clinical & Biochemical Research Regarding the Etiology of Idiopathic Steatorrhea* (Paper presented at the Intern. Congr. Gastroenterology, Leiden, Netherlands, April, 1960)
98. Borgström, B., and Lindquist, B., *Acta Paediat.*, 46, 449 (1957)
99. Lindquist, B., and Rafstedt, S., *Acta Paediat.*, 48, 50 (1959)
100. Rubin, C. E., Brandborg, L. L., Flick, A. L., Parmentier, C., Phelps, P. C., and van Niel, S., *J. Clin. Invest.*, 39, 1023 (1960)
101. Sleisenger, M. H., Law, D. H., Kowlessar, O. D., Pert, J. H., and Almy, T. P., *Trans. Assoc. Am. Physicians*, 71, 100 (1958)
102. Ebbs, J. H., Thompson, M., and Stein, W. O., *Am. J. Diseases Children*, 79, 936 (1950)
103. Thompson, M. W., *Am. J. Human Genet.*, 3, 159 (1951)
104. Boyer, P. H., and Anderson, D. H., *Am. J. Diseases Children*, 91, 131 (1956)
105. Carter, C., Sheldon, W., and Walker, C., *Ann. Human Genet.*, 23, 266 (1959)
106. Grasbeck, R., Gordin, R., Kantero, I., and Kuhlback, B., *Acta Med. Scand.*, 167, 289 (1960)

CARDIOVASCULAR DISEASE: MYOCARDIOPATHIES[1]

By Eugene D. Robin, M.D.
Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

This review will attempt to develop some broad general concepts regarding the myocardiopathies rather than being exhaustive or complete. The references cited are generally limited to the English language.

Scope of This Review

Approximately 25 years ago, chronic myocarditis was a frequent and popular diagnosis. It was defined as a lesion in which cardiac failure was associated with normal valves and pericardium while the heart muscle showed hypertrophy alone or in combination with fibrous interstitial myocarditis (1). Subsequent work established that most of these patients had either hypertensive heart disease or coronary artery disease as a cause of their cardiac failure. As a result, the diagnosis of chronic myocarditis received less and less attention. In recent years, the importance of diseases involving the myocardium, more or less in specific fashion, has become apparent and it is now generally accepted that a large number of unrelated diseases may affect the myocardium. Saphir has been a leader in defining this group of diseases and has carefully documented their nature and incidence (2). He has adopted the generic term "myocarditis" to describe them. However, this term is not completely satisfactory since many of these diseases are not basically inflammatory. The term "myocardosis" has also been suggested (3) but this designation is likewise unsatisfactory because of the inflammatory nature of a number of the entities comprising this disease category.

The term "myocardiopathy" has therefore been selected to describe this group of diseases. The myocardiopathies may be defined as a broad group of diseases of diverse etiology that specifically involve the myocardium to produce abnormalities of structure, abnormalities of function, or both. The end result of many of these disease processes may be the development of myocardial fibrosis.

Functional Pathology

There are various mechanisms by which the abnormalities of structure related to a given myocardiopathy may become translated into functional abnormalities.

One such mechanism is acute massive necrosis of myofibers. Acute destruction of muscle fibers may be so extensive that the remaining muscle fibers are simply not adequate in amount to maintain a normal cardiac output (4). In addition to the quantitative insufficiency of muscle, the reduction

[1] The survey of the literature pertaining to this review was concluded in September, 1960.

of coronary blood flow associated with the reduced cardiac output may damage previously uninvolved fibers. Even if the degree of necrosis is not sufficient to precipitate failure quickly, the work load of uninvolved myofibrils is necessarily increased, resulting in ultimate hypertrophy and the ultimate development of failure.

In other forms of myocardiopathy (infectious myocarditis, isolated myocarditis, hypersensitivity myocarditis), the inflammatory infiltrate may be limited to the interstitial tissues of the heart with little or no evidence of direct injury to contractile elements (4). The mechanism of cardiac insufficiency is not completely clear in such cases. However, one factor which seems to be of importance, under the circumstances, is the impairment of diastolic filling and systolic emptying resulting from the distortion by the infiltrate of the normal volume-pressure characteristics of the heart. This mechanism is clearly present in patients with diffuse myocardial fibrosis (5).

Another mechanism for the development of cardiac failure is the compression effect of diffuse myocardial infiltrations. For example, in some forms of cardiac amyloidosis individual myofibrils are surrounded, compressed and ultimately undergo atrophy as the quantity of amyloid increases (6, 7, 8). The relationship between myocardial atrophy and cardiac failure has clearly been shown in some patients with constrictive pericarditis (9).

Another mechanism that contributes to the development of cardiac failure is related to situations in which there is a deficiency in, or an inability to use, oxygen or substrate. A priori, one would expect cardiac muscle to be particularly vulnerable to such varieties of injury because of the high myocardial energy requirements as shown by the relatively high proportion of total cardiac output utilized by the myocardium (10); as shown by the high degree of oxygen extraction by the myocardium (11); and as shown by the fact that myocardial cells have a far greater number of mitochondria than other muscle cells (12). Such divergent diseases as beri-beri, shock, and anemia may lead to hydropic degeneration, myofibrillar swelling, and finally to frank myocardial necrosis (4). Ultimately, it may be that many or all of the etiologic factors capable of producing the myocardiopathies fundamentally interfere with the normal energetics of cardiac muscle.

Finally, a given process may produce its manifestations by involving the conduction apparatus of the heart. In the myocardiopathies, the entire gamut of conduction and rhythm disturbances may be seen including partial, intraventricular and complete heart block, A-V dissociation, atrial, nodal, and ventricular ectopic beats or tachycardias. Such disturbances may occur in the absence of histologically demonstrable lesions of the conduction system and, conversely, there may be histologic abnormality in these areas without conduction or rhythm disturbance (13, 14).

Etiologic Background

The etiologic background of the myocardiopathies is vast. A partial list of the etiological situations underlying the myocardiopathies is shown in Table I. Any classification of this variety is, to a certain extent, arbitrary

TABLE I

THE ETIOLOGIC BACKGROUND OF THE MYOCARDIOPATHIES

- I. Myocardiopathy associated with infection (infectious myocarditis)
  - A. Bacterial
    - Diphtheria
    - Suppurative
  - B. Viral or postviral
    - Coxsackie
    - Influenza (15, 16, 17)
    - Poliomyelitis (18, 19)
    - Measles (20)
    - German measles (21)
    - Mumps (22)
    - Chicken pox (23)
    - Smallpox (24)
    - Viral pneumonia (25)
    - Interstitial pneumonia with myocarditis (26)
    - Viral hepatitis (27)
    - Infectious mononucleosis (28)
    - Encephalitis with myocarditis (25)
    - Encephalomyocarditis (EMC virus) (29)
  - C. Granulomata of infectious etiology
    - Tuberculosis (30)
    - Syphilis (30)
    - Fungal
  - D. Parasitic
    - Trichinosis
    - Schistosomiasis (31)
  - E. Protozoan
    - Chagas disease (32)
    - Toxoplasmosis (33, 34)
    - Amebic (28)
  - F. Leptospira (35)
  - G. Rickettsial (36)
- II. Myocardiopathy associated with systemic inflammatory disease
  - A. "Collagen" diseases
    - Rheumatic fever
    - Acute glomerulonephritis
    - Lupus erythematosus
    - Polyarteritis nodosa
    - Scleroderma
    - Dermatomyositis
    - Rheumatoid arthritis
  - B. Sarcoidosis
- III. Myocardiopathy associated with non-inflammatory systemic disease
  - A. Amyloidosis
  - B. Hemochromatosis
  - C. Hemosiderosis
- IV. Myocardiopathy associated with metabolic or nutritional disorders
  - A. Beri-beri
  - B. Xanthomatosis
  - C. Glycogen storage disease (37)
  - D. Fat storage disease (37)
  - E. Endomyocardial fibrosis
  - F. Anemia
- V. Myocardiopathies associated with hypersensitivity reactions
  - A. Serum sickness
  - B. Penicillin
  - C. Arsphenamine
  - D. Sparteine
  - E. Bismuth
  - F. Sulfonamides
  - G. Phenylbutazone
- VI. Toxic myocardiopathies
  - A. Chloroform
  - B. Phosphorus
  - C. Carbon monoxide
  - D. Benzol
  - E. L-Norepinephrine
- VII. Myocardiopathies associated with muscle or neuromuscular disease
  - A. Myotonia atrophica
  - B. Friedreich's ataxia
  - C. Progessive muscular dystrophy
  - D. Myasthenia gravis
  - E. Thymoma
  - F. Myocardiopathy associated with smooth muscle myositis
  - G. Myocardiopathy associated with skeletal muscle myositis
  - H. Thyrotoxicosis
- VIII. Obscure
  - A. Fiedler's myocarditis
  - B. Giant cell myocarditis
  - C. Congenital idiopathic hypertrophy (38)
  - D. Adult idiopathic hypertrophy (39)
  - E. Chronic fibroplastic myocarditis (40)
  - F. Idiopathic right cardiac hypertrophy (41)

but has the virtue of stressing important inter-relationships. Where discussion of a given entity is not undertaken in the text, suitable references are cited in the table.

The best characterized and understood myocardiopathies are those related to infection. Infectious agents produce disorders of myocardial function in a variety of ways. There may be direct invasion of the myocardium by organisms with destruction of fibers produced by liberated endotoxin such as in suppurative myocarditis.

The organism may elaborate an exotoxin which interferes with myocardial metabolism. This is presumably the mechanism by which diphtheric myocarditis is produced. It has been suggested that diphtheria toxin produces histologic damage and cardiac failure by interfering with the normal function of cytochrome-*b* since diphtheria exotoxin closely resembles in structure the protein fraction of this respiratory enzyme (42). If the oxidation of succinate is blocked by diphtheria toxin then increased levels of succinate dehydrogenase levels might be present in affected muscles. Recent histochemical studies by Mustakallio indicate that this is the case (43).

Viral agents may invade either nucleus or cytoplasm of myocardial cells and produce perversions of cellular metabolism resulting in abnormal myocardial function (44).

Finally, the original effect of the infectious agent upon the myocardium may be relatively unimportant, but the reaction of the myocardium to the presence of the infectious agent may result in myocardial damage. For example, in trichinous myocarditis, the organism is rarely found in the myocardium and the myocarditis appears to depend on an allergic or hypersensitivity reaction (45). This is presumably also the mechanism of the postviral myocarditides (24).

Myocarditis as shown by clinical or pathological criteria has been described as an occasional complication of most viral diseases. In particular, the development of transient electrocardiographic abnormalities has been used to document the existence of myocardial involvement.

However, during the past 10 years, there has been the emergence of practical and reliable techniques for the diagnosis and isolation of specific virus agents (46). Such techniques have permitted more specific delineation of the role of viruses in the pathogenesis of myocarditis. An excellent example of the use of these techniques concerns the relationship between the Coxsackie group of viruses and myocarditis.

It has been shown that Group B Coxsackie viruses may be associated with a fatal myocarditis of newborn human infants (47 to 57). It has also been shown that this group of viruses may produce pericarditis or myocarditis, or both, in the adult human (58 to 65). Myocarditis has also been produced experimentally in young and adult mice by exposure to Coxsackie virus (66, 67, 68).

Human prenatal infection is severe and frequently fatal. The primary

sites of infection are the myocardium, brain, and meninges. Virus has been recovered from feces (69, 70), brain (50), spinal cord (51), and myocardium (50, 57, 70, 71) of patients. The evidence for transplacental transmission is convincing (52).

Adult mice and humans are much more resistant to the infection than are young animals. With some strains, exposure to cold or pretreatment with cortisone is required to produce infection of adult mice. Adult human myocarditis is relatively uncommon as compared with its incidence in the newborn (53). The basis for the increased susceptibility of the newborn is not clear.

In addition to patients with obvious myocardial involvement, it seems likely that subclinical forms of infection are common. The relationship between subclinical infection and chronic heart failure of unknown etiology is conjectural.

Aside from its direct clinical importance, Coxsackie virus myocarditis may be regarded as a model for future studies. As basic knowledge concerning viruses increases, and as specific methods for viral isolation and viral diagnosis improve, the exact relationship between the various virus diseases and myocarditis will become clearer.

As mentioned above, the time relations involved in some of the postviral myocarditides and, in particular, the development of myocarditis following vaccination (23), suggests that hypersensitivity factors are important in the pathogenesis of these forms of myocarditis.

In recent years there has been an apparent increase in the incidence of fungal myocarditis (28). Such fungal agents as *Candida* and *Aspergillus* as well as fungi of greater inherent pathogenicity, have been reported as causative agents. Some of this increased incidence is undoubtedly related to the increased use of antibiotics (28). Depending on the fungal agent, there may be the development of a necrotizing myocarditis or a granulomatous myocarditis (29).

The myocardiopathies associated with the so-called collagen diseases present a broad spectrum of disorders. There is a wide difference in the degree and type of myocardial involvement. In rheumatic fever, the heart appears to be a primary focus of involvement, whereas myocardial involvement, when present, in rheumatoid arthritis is almost incidental. Also, there are wide individual variations in the degree of myocardial involvement in any specific member of this group. Myocardial involvement in scleroderma with the development of diffuse myocardial fibrosis is probably the best characterized member of this group. Whether there is any unitary pathogenetic basis in this group of diseases remains to be established.

Sarcoidosis is a disease of unknown etiology whose incidence is apparently increasing (72). Myocardial involvement occurs in approximately 5 to 10 per cent of patients (73). Myocardial sarcoidosis in the absence of involvement of other organs has not been reported (29). Involvement takes the form

Friedreich's ataxia (88), progressive muscular dystrophy (89), postmenopausal muscular dystrophy (90), myasthenia gravis (91), thyrotoxicosis and thyrotoxic myopathy (92), thymoma in the absence of myasthenia gravis (93), generalized skeletal muscle myositis (94), and with smooth muscle myositis (95).

The final group of myocardiopathies are obscure in terms of their pathogenesis. The most important members of this group are Fiedler's myocarditis and giant cell myocarditis.

Fiedler's myocarditis is an acute interstitial disease. It is generally regarded as an isolated myocarditis, that is, a myocarditis occurring without involvement of any other organs. It is not clear that this represents a single disease entity. It has been reported to occur in epidemic fashion (96) and the possibility of a viral etiology has been raised. On the other hand, sporadic cases are more common and an allergic basis for some of these has been postulated on the basis of some rather tenuous evidence (97). Clinically, the disease is associated with a tendency for sudden and unexpected death.

Giant cell myocarditis presents a picture which clinically resembles Fiedler's variety but, histologically, the interstitial infiltrate contains varying numbers of giant cells (29). The differentiation between this variety of myocarditis and Fiedler's is one of only pathological interest.

Hemodynamic Abnormalities

The hemodynamic abnormalities found in diffuse myocardial disease show a relatively consistent pattern. This form of heart disease is associated with a low cardiac output that results, in part, from a restriction of diastolic filling of the heart. This restriction is shown by the presence of a pressure plateau from the peripheral veins to the pulmonary capillaries (mean pressure in peripheral veins, right atrium, right ventricular end diastolic pressure, pulmonary artery diastolic pressure, and mean pulmonary capillary pressure do not differ by more than 5 mm. of Hg). Such a pressure plateau is also present in patients with constrictive pericarditis and appears to result from an alteration of the normal volume-pressure distensibility characteristics of the heart. In the case of myocardial disease, it appears that the infiltrating material imposes its own distensibility characteristics on the myocardium. In the case of constrictive pericarditis, the thick, fibrous connective tissue making up the pericardial scar plays the same role.

The absence of a pressure plateau in some patients (relatively high pressures in the right ventricle, pulmonary artery, and pulmonary capillaries) is found in patients with primarily left ventricular involvement, evidence that is consistent with the thesis of distensibility alteration as the fundamental defect.

Impairment of systolic emptying is presumably present as well, although evidence for this is histological and therefore indirect. The fact that diffuse

groups (103, 104) as a diagnostic aid in heart disease of unknown etiology without mortality or serious morbidity. This technique is too recent for critical appraisal.

One is therefore left with a careful history, physical examination, radiologic examination, and electrocardiogram as the best general diagnostic measures available for the study of patients with the myocardiopathies and, from this point, one must proceed with a knowledge of the differential diagnosis in an attempt to establish a more specific diagnosis.

LITERATURE CITED

1. Christian, H. A., *New Engl. J. Med.*, 208, 574 (1933)
2. Saphir, O., *Arch. Pathol.*, 32, 1000 (1951)
3. De la Chapelle, C. E., and Kossmann, C. E., *Circulation*, 10, 747 (1954)
4. Kuschner, M., and Lobdell, D. H., *J. Chronic Diseases*, 9, 424–41 (1959)
5. Robin, E. D., and Burwell, C. S., *Circulation*, 16, 730–35 (1957)
6. Lindsay, S., *Am. Heart J.*, 32, 419–37 (1946)
7. Eisen, H. H., *Am. J. Med.*, 1, 144–60 (1946)
8. Hetzel, P. S., Wood, E. H., and Burchell, H. B., *Proc. Staff Meetings, Mayo Clinic*, 28, 107 (1953)
9. Sawyer, C. G., Burwell, C. S., Dexter, L., Eppinger, E. C., Goodale, W. T., Gorlin, R., Harken, D. E., and Haynes, F. W., *Am. Heart J.*, 44, 207 (1952)
10. Bing, R. J., Vandam, L. D., Gregoire, F., Handelsman, J. C., Goodale, W. T., and Echenhoff, J. E., *Proc. Soc. Exptl. Biol. Med.*, 66, 239 (1947)
11. Bing, R. J., *Circulation*, 12, 635 (1955)
12. Kisch, B., *Exptl. Med. Surg.*, 13, 404 (1955)
13. Herman, R. H., Scripter, L. J., and Mattingly, T. W., *Am. Heart J.*, 57, 829 (1959)
14. Tlusty, T., *Brit. Med. J.*, 21, 145–48 (1959)
15. Silber, E. N., *Ann. Internal Med.*, 48, 228–41 (1958)
16. Bowden, K. M., and French, E. L., *Med. J. Australia*, 1, 553–56 (1958)
17. Oseasohn, R., Adelson, A., and Kaji, M., *New Engl. J. Med.*, 260, 509–18 (1959)
18. Marinesco, G., Turco, T., Friedman I., Ciureze, V., and Draganesco, N., *Semaine hôp. Paris*, 33, 212 (1957)
19. Wolvius, D., Deenstra, H., and Wagenvoort, C. A., *Ann. Paediat.*, 187, 113 (1956)
20. Neubauer, C., *Arch. Diseases Childhood*, 19, 178 (1944)
21. Logue, B. L., and Hanson, J. L., *Am. Heart J.*, 30, 215 (1945)
22. Horton, G. E., *Ann. Internal Med.*, 49, 1228–39 (1958)
23. Sampson, C. C., *J. Natl. Med. Assoc.*, 51, 138–39 (1959)
24. Bengtsson, E., and Lundstrom, R., *Cardiologia*, 30, 1 (1957)
25. Saphir, O., and Cohen, N. A., *Arch. Pathol.*, 64, 446–56 (1957)
26. Morrow, D. F., and Coady, C. J., *Can. Med. Assoc. J.*, 80, 980–82 (1959)
27. Saphir, O., Amromin, G. D., and Yokoo, H., *Am. J. Med. Sci.*, 231, 168–76 (1956)
28. Fish, M., and Barton, H. R., *Arch. Internal Med.*, 101, 636–44 (1958)
29. Saphir, O., *Am. Heart J.*, 57, 639–42 (1959)
30. Dilling, N. V., *J. Pathol. Bacteriol.*, 71, 295–300 (1956)
31. Zahawi, S. A., and Shukri, N., *Trans. Roy. Soc. Trop. Med. Hyg.*, 50, 166–68 (1956)
32. Decourt, L. V., Ramos, J., Trancheri, B., Corea, I. A., Dias, C., and Tisi, G., *Am. Heart J.*, 33, 697–98 (1947)
33. Potts, R. E., and Williams, A. A., *Lancet*, I, 483–84 (1956)
34. Hakkila, J., Frick, H. M., and Halonen, P. I., *Am. Heart J.*, 55, 758–65 (1958)
35. Arean, V. M., *Lab. Invest.*, 6, 462–71 (1957)
36. Pappenheimer, A. M., *J. Natl. Cancer Inst.*, 20, 921–31 (1958)
37. Fisher, E. R., and Davis, E. R., *Am. Heart J.*, 56, 537–52 (1958)
38. Kugel, M. A., *Am. Heart J.*, 17, 602 (1939)
39. Levy, R. L., and von Glahn, W. C., *Trans. Assoc. Am. Physicians*, 52, 259 (1937)
40. Ware, E. R., and Chapman, B. M., *Am. Heart J.*, 33, 530 (1947)
41. Rosenbaum, F. F., *Ann. Internal Med.*, 26, 76 (1947)
42. Pappenheimer, A. M., Jr., and Williams, C. M., *J. Gen. Physiol.*, 35, 727 (1952)
43. Mustakallio, K. K., *Exptl. Cell Research*, 7, 592 (1954)
44. Horsfall, F. L., Jr., *Am. Rev. Respiratory Diseases*, 80, 315–25 (1959)
45. Chase, G. O., *J. Am. Med. Assoc.*, 165, 1826–29 (1957)
46. Enders, J. F., *Ann. Internal Med.*, 45, 331–50 (1956)
47. Van Creveld, S., and DeJager, H., *Ann. Paediat.*, 187, 100–12 (1956)
48. Delaney, T. B., and Fukunaga, F. H., *New Engl. J. Med.*, 259, 234–36 (1958)

49. Hosier, D. M., and Newton, W. A., *J. Diseases Children*, 96, 251–67 (1958)
50. Kagan, H., and Bernkopf, H., *Ann. Paediat.*, 189, 44–50 (1957)
51. Kibrick, S., and Benirschke, K., *New Engl. J. Med.*, 255, 883–89 (1956)
52. Kibrick, S., and Benirschke, K., *Pediatrics*, 22, 857–75 (1958)
53. Naude, W. Du T., Selzer, G., and Kipps, A., *Med. Proc.*, 4, 397–401 (1958)
54. Rapmund, G., Gauld, J. R., Rogers, N. G., and Holmes, G. E., *New Engl. J. Med.*, 260, 819–21 (1959)
55. Simenhoff, M. L., and Uys, C. J., *Med. Proc.*, 4, 389–97 (1958)
56. Suckling, P., and Vogelpoel, L., *Med. Proc.*, 4, 372–89 (1958)
57. Verlinde, J. D., Van Tongeren, H. A. E., and Kret, A., *Ann. Paediat.*, 187, 113–18 (1956)
58. Fletcher, E., and Brennau, C. F., *Lancet*, I, 913–15 (1957)
59. Fletcher, E., and Brennan, C. F., *Lancet*, II, 585 (1958)
60. Kagan, H., and Bernkopf, H., *Ann. Paediat.*, 189, 44–50 (1957)
61. Movitt, F. R., Lennette, E. H., Mangum, J. F., Berk, M., and Bowman, M. S., *New Engl. J. Med.*, 258, 1082–86 (1958)
62. McLean, D. M., Walker, S. J., and Bain, H. W., *Can. Med. Assoc. J.*, 79, 789–93 (1958)
63. Roberts, R., Lydon, M., and MacIntosh, M., *Can. Med. Assoc. J.*, 80, 722–25 (1959)
64. Varcasia, E., and Castelli, L., *Rend. ist. super. sanità, Suppl. 1*, 20, 831–41 (1957)
65. Weinstein, S. B., *New Engl. J. Med.*, 257, 265–67 (1957)
66. Gifford, R., and Dalldorf, G., *Am. J. Pathol.*, 27, 1047–63 (1951)
67. Walker, D. L., and Boring, W. D., *Research Rept. U. S. Naval Med. Research Inst., Bethesda*, 15, 815–24 (1957)
68. Grodums, E. I., and Dempster, G., *Can. J. Microbiol.*, 5, 605 (1959)
69. Montgomery, J., Gear, J., Prinsloo, F. R., Kahn, M., and Kirsch, Z. G., *S. African Med. J.*, 29, 608 (1955)
70. Van Creveld, S., and DeJager, H., *Ann. Paediat.*, 187, 100 (1956)
71. Gear, J., Measroch, V., and Prinsloo, F. R., *S. African Med. J.*, 30, 806 (1956)
72. Cummings, M. M., Dunner, E. D., and Williams, J. H., Jr., *Ann. Internal Med.*, 50, 879–90 (1959)
73. Longcope, W. T., and Freiman, D. G., *Medicine*, 31, 1–132 (1952)
74. Hardy, H. L., *Am. Rev. Tuberc. Pulmonary Diseases*, 74, 885–96 (1956)
75. Ballinger, J., *Am. J. Med. Sci.*, 217, 308–13 (1949)
76. Atkinson, F. R. B., *Med. Press*, 195, 312–27 (1937)
77. Josselson, A. J., Pruitt, R. D., and Edwards, J. E., *Med. Clin. N. Am.* 1137–44 (1950)
78. Bothwell, T. H., Lingen, B. V., Alper, T., and Du Preez, M. L., *Am. Heart J.*, 43, 333–40 (1952)
79. Levin, E. B., and Golum, A., *Am. Heart J.*, 45, 277 (1953)
80. Gellerstedt, N., *Acta Pathol. Microbiol. Scand.*, 16, 386 (1939)
81. Glanzmann, E., and Walthard, B., *Mschr. kinderheilk.*, 88, 1 (1941)
82. Campbell, S., and Macafee, C. A. J., *Arch. Diseases Childhood*, 34, 218–22 (1959)
83. Hodge, P. R., and Lawrence, J. R., *Med. J. Australia*, 1, 640–41 (1957)
84. Szakacs, J. E., and Cannon, A., *Am. J. Clin. Pathol.*, 30, 425–34 (1958)
85. Mond, E., and Mack, I., *Am. Heart J.*, 59, 134–39 (1960)
86. Cannon, P., and Sjostrand, T., *Acta. Med. Scand.*, 146, 191–208 (1953)
87. Evans, W., *Brit. Heart J.*, 6, 41 (1944)
88. Russell, D. S., *J. Pathol. Bacteriol.*, 58, 739 (1946)
89. Moore, W. F., Jr., *J. Pediat.*, 44, 683 (1954)
90. Waller, J. V., Shapiro, M., and Paltauf, R., *Am. Heart J.*, 53, 479–84 (1957)
91. Mendelow, H., and Genkins, G., *J. Mt. Sinai Hosp.*, 21, 218 (1954)
92. Terplan, K. L., Constantine, A. B., Koepf, G. F., and Dayton, G. O., *New York J. Med.*, 51, 2750 (1951)
93. Langston, J. D., and Dickenman, R. C., *Arch. Pathol.*, 68, 367–73 (1959)
94. Adams, R. D., Denny-Brown, D., and Pearson, C. M., *Diseases of Muscle: A Study in Pathology*, 152 (Paul B. Hoeber, Inc., New York, N. Y., 1953)
95. Ramos, H. S., Scalettar, R., Martz, D. G., and Mattingly, T. W., *Am. Heart J.*, 57, 395–406 (1959)
96. Freundlich, E., Berkowitz, M., Elkon,

A., and Wilder, A., *J. Diseases Children*, 96, 43–50 (1958)
97. Lieberman, A., *Geriatrics*, 12, 485–88 (1957)
98. Burwell, C. S., and Robin, E. D., *Circulation*, 20, 606–14 (1959)
99. Clark, G. M., Valentine, E., and Blount, S. G., Jr., *New Engl. J. Med.*, 254, 349 (1956)
100. Balchum, O. J., McCord, M. C., and Blount, S. G., Jr., *Am. Heart J.*, 52, 430–33 (1956)
101. Chesler, E., *Brit. Heart J.*, 20, 244–48 (1958)
102. Wróblewski, F., Ross, C., and Gregory, K., *New Engl. J. Med.*, 263, 531–36 (1960)
103. Sutton, D. C., and Sutton, G. C., *Am. Heart J.*, 60, 364–70 (1960)
104. Fremont, R. E., Losner, S., and Volk, B. W., *Arch. Internal Med.*, 102, 41–49 (1958)

CARDIOVASCULAR DISEASE: EXTRACORPOREAL CIRCULATION[1]

By D. G. Melrose, M.A., B.M.B.Ch.

Departments of Clinical Physiology, Postgraduate Medical School and Hammersmith Hospital, London, England

In 1953, Gibbon (1) reported the repair, under direct vision, of an interatrial septal defect. The patient, an adult woman, recovered completely. The normal circulation was excluded from the heart for 25 min., the heart's function and that of the lungs being imitated successfully by a heart-lung machine. This report confirmed the application to man of many years of speculation and experimentation, and ushered in a new era of surgery.

In 1812, Le Gallois (2) had written "If one could substitute for the heart a kind of injection of arterial blood, either naturally or artificially made, one would succeed in maintaining alive indefinitely any part of the body whatsoever." In 1858, Brown-Sequard (3) performed such experiments. After the decapitation of a dog, cannulae were inserted into the common carotid and vertebral arteries on both sides and arterial blood injected by means of a syringe. Movements of the eyes and jaws continued during the 15 min. of perfusion. Earlier, this great experimentalist had confirmed that cessation of flow in these vessels for longer than 5½ min. caused the death of the dog. Ten years later, Ludwig (4) and Schmidt reported an attempt to oxygenate venous blood in their perfusion apparatus by shaking it in a balloon. The method was not effective enough to permit a continuous perfusion. In 1882, Shröder (5) found that he could not effectively oxygenate venous blood by bubbling air through it without converting a large proportion of it into foam. Jacobj (6) in 1890 and Brodie (7) in 1903 mixed air and blood by pumping them through a tube.

A different principle was used by Frey & Gruber (8) in their artificial lung built in 1885. They spread blood in a thin film over the inner surface of a cylinder and exposed it to oxygen. The surface area was about half a square metre. Several variants of these methods were tried in the succeeding years and so ineffective were they that many physiologists abandoned artificial methods entirely and made use of animal heart-lung preparations.

In 1929, Brukhonenko (9), on the basis of several years of animal experiments, was able to conclude:

> Would not this method, duly perfected, be useful in clinical medicine; notably in those cases where it would be essential to replace, if only for a time, the work of the failing human heart? Without going more deeply into this question we can state as a result of the present work, that, in principle artificial circulation is applicable to man not only clinically, but perhaps also for certain operations on the temporarily arrested heart. For its achievement, however, a suitable technique would have to be worked

[1] The survey of the literature pertaining to this review was concluded in July, 1960

out. The solution of the problem of the artificial circulation of the whole animal opens the door to the problem of operations on the heart, for example on the valves.

One other contribution prior to 1953 stands out and that was the detailed description by V. O. Björk in 1948 (10) of his work on perfusion of the brain. This is a landmark for two reasons—it was the first full description of the rotating disc oxygenator, whicb is now the most successful device in clinical use, and it contained a masterly analysis of the problems to be encountered.

Work reported in several papers between this date and 1955 (11 to 19) helped to lay a firm experimental basis for the clinical application of the heart-lung machine which is now the dominant force behind advances in cardiac surgery. It is this clinical application that has, deservedly, commanded most of the energy in this field and it is not surprising that mechanical design has lagged somewhat.

Pumping Systems

Little has been added to the specifications of an ideal pump introduced at the work conference held in Chicago in September, 1957 (20) and the literature contains few references to new pumps.

One model of interest is the Mono pump described by Hall *et al.* (21). This pump is novel in that the flow from it is continuous in type. It works by the rotation of a screw within an elastic stator of different pitcb, so that the fluid is drawn along the thread as the rotor revolves. Friction is, of course, generated between the two members of the pump, wbich should be destructive, but in tests it bas been surprisingly atraumatic. Its use in the pumping of strawberry jam without destroying the berries perhaps demonstrates this aspect of it. Recently used clinically in England, it may well join the more conventional patterns.

The author (22) has modified the classical roller pump of DeBakey to allow plastic tubing of normal form to be used more satisfactorily. The modification consists of grooving the backplate to accept the tube along which runs a roller, the shape of which is such that it invaginates one wall of the tube into the other. If the radii of roller and groove are arranged to match one another, an efficient occlusion is produced with minimum trauma. A further modification is that the rollers are carried on spring-loaded shafts so the tension may be adjusted to provide optimum efficiency. That degree of tension which maintains a constant output at a predetermined resistance provides the most satisfactory way of ensuring accurate perfusion.

Too little attention has been directed to the problem of clearing blood from the open heart. Miller & Albritten (23) have published an account of experiments designed to show how serious a problem this can be. They showed in dogs how the degree of negative pressure used to withdraw blood can influence perfusion with a Kay Cross oxygenator. A high negative pressure predisposes to air embolism by entraining minute bubbles.

The method advocated by Donald, Harshbarger & Kirklin (24) whereby

a roller pump withdraws blood by its pumping action, is favoured by the author who believes that two such suction lines are necessary as well as one of the negative pressure type. A modification of their method in which blood so pumped is allowed to degas in a helix system similar to that of DeWall (25) aids in the elimination of air.

Oxygenators

Three new oxygenators have been announced, one of which is of novel type. This is a combination of the rotating drum and membrane systems (26, 27, 28) whereby blood is streamed over a rotating membrane of siliconized nylon through which gas diffuses into and out of the blood. Thomas (29) has described it and claimed a very high efficiency for it. Certainly, it seems a useful step towards the development of a wholly satisfactory membrane system. Some of the factors implicit in such a device have been discussed by Benvenuto & Lewis (30). Such a mechanism must have a great deal of appeal although until development proceeds to the point where packaged units can be drawn from store, the full value will not have been realised.

The membrane itself must have very special properties (31), allowing diffusion of oxygen and carbon dioxide through it at a rate of about 20 cc./sq.m./min. without itself being so porous that blood leaks through it. It must have sufficient mechanical strength to withstand pressure, be capable of sterilisation by heat, and be inert in respect to blood—at first sight an almost impossible ideal. However, chemical engineers have found that silicone elastomers can be produced that virtually meet the specification. At this time the problem has advanced from a search for a perfect membrane to the problem of its best utilisation.

The provision of a wholly suitable semipermeable membrane does not in itself solve the many problems of design encountered in an oxygenator of this type. The membrane must necessarily be supported in a manner which provides sufficient rigidity and protection and allows the maximum of surface area to be utilised. Unsupported membrane layers do not provide for an even distribution of blood on their surfaces, and the formation of rivulets and streams results in only partial use of the available surface. A practical solution is to sandwich a pair of membranes between two plates whose surface is corrugated. If the grooves in one plate are set at an angle to those in the other, then a quilted effect is produced on the membranes between them and excellent distribution of blood results. By trial and error, a matching of characteristics can be achieved wherein such a quilt will allow free blood flow at a minimal pressure gradient without trapping a large quantity of blood between the films.

Having thus achieved an effective spreading of thin films of blood between adjacent membranes, another factor comes into play. There is on the surface of the membrane a layer of fluid known as the boundary layer; if this is not disturbed and broken up it acts as a barrier to the full efficiency of gas

diffusion. The natural solution is the rhythmic pulsing of respiration in the lung which must, in part, disturb this layer. An imitation of this effect could be achieved in the membrane-oxygenator.

If a pulsing effect can be given to the lung then it is logical to attempt to use the lung itself as a circulating pump. If two sections of a membrane-oxygenator are set up so that they can be expanded and contracted alternately, and if the inlet and outlet to each section are equipped with valves, then a combined oxygenator and pump is created. With the addition of accurate heat control a heart-lung machine very close to the ideal will be available. Such a device has been made to function but as yet many problems remain unsolved (32).

Perhaps the most widely used oxygenator is that employing rotating discs and that of Kay and Cross the most popular model. They have improved the performance of their device by using convoluted discs in place of the smooth ones previously specified (33). However, it is a disc oxygenator of quite new form that is the second of the new types. Osborn *et al.* (34) have described their modification which has considerable advantages over the conventional type. With greatly increased efficiency of oxygenation they have combined a useful heat exchanger to make a very elegant improvement.

Many and various are the bubble oxygenators. An interesting development of this basic system is the third of the new types. It is described by Panico & Neptune (35) and has, as its chief advantage, the facility of being worked without being primed with blood. Such an attribute is, of course, of very considerable significance and much interest attends its clinical trials. The ever-increasing demands for donor blood in cardiac surgery are imposing a large burden on the community and this characteristic of priming volume will play a vital part in judging the merits of any heart-lung machine.

Management of Perfusion at Normal Body Temperature

Now that the controversy between the low flow and high flow principles is at an end, it is possible to be rather more specific as to what constitutes an adequate perfusion, and, indeed, little need to be added to the comments of Kirklin *et al.*, Clark, and Varco *et al.* in the monograph, *Extracorporeal Circulation* (20).

The author subscribes to the view that a satisfactory perfusion is one which is physiologically undetected by the patient, and suggests that preservation of a constant pressure in the central venous system does much to eliminate vascular readjustments (36). Considerable clinical experience with a regimen that places emphasis on an arbitrary but fixed arterial input at 2.4 l/min./sq.m., and a constant central venous pressure only underlines its usefulness.

For its successful use, it is essential to have available an adequate range of sizes of both arterial and venous cannulae and to know the performance of these various tubes (37). Table I is a synopsis of such an analysis and forms

TABLE I

A Guide to Normothermic Perfusions on the Basis of Surface Area of Patients

Surface Area in sq. m.	Output from Pump, cc.	Int. Dia. of Cannula, mm.	Pressure Created by Cannula, mm.Hg	Venous Catheter Int. Dia. mm.
0.4	960	2.5	100	3.9
0.5	1200	3	60	4.7
0.6	1440	3	75	4.7
0.7	1680	3	100	4.7
0.8	1920	3.5	75	5.5
0.9	2160	3.5	100	5.5
1.0	2400	4	70	5.5
1.2	2880	4	80	6.3
1.4	3360	4.5	75	6.3
1.6	3840	4.5	100	6.3
1.8	4320	5	100	7.9
2.0	4800	5.5	90	7.9
2.2	5280	6	85	7.9

an effective guide to perfusions on the basis of the surface area of patients. A boon conferred by the choice of appropriate venous catheters is that the venous pressure will be but rarely affected by their emplacement in the venae cavae. Thus, it will not be important to hurry the initiation of perfusion in order to avoid the fall in cardiac output that follows obturation of the venae cavae by too large a catheter, nor will it be necessary to suck hard on these tubes to obtain the required flow.

Having placed into the venae cavae tubes of the predicted size, it is possible to adjust the venous return in either of two ways. Either the suction pressure on the catheters is varied by altering the height difference between the patient and the heart-lung machine or, more simply, a throttle is adjusted on the venous line. In this manner, the pressure within the great veins is maintained at a constant level and, in doing so, it will be found that the patient's blood volume remains constant as does the balance between arterial inflow and venous return. Any change in level of blood in the heart-lung machine is caused by an alteration in the volume of blood obtained in the combined system of patient and machine. It may be loss from the operation site; it may be merely a reflection of the volume of blood contained witbin the heart and lungs themselves. The increment to the circulating blood volume, when a large heart is emptied, may exceed a litre and, conversely, when such a heart is refilled the decrement may be as large. Allowance is properly made for such changes, not by altering the perfusion rate as is likely

when the venous return controls the arterial rate, but by temporarily storing this blood until it is again required.

Thus, the perfusion proceeds with no alteration in rate of arterial input and, by the maintenance of a constant venous pressure, allows automatically for alterations in combined blood volume, seeking always to preserve that of the patient rather than that of the machine. In such a manner, it is possible to ignore the machine save to prevent it from running too empty for safety and to complete the perfusion without reference to the sometimes complex distribution of blood in various reservoirs, tubes, and other apparatus. In like manner, it is possible to ignore the characteristic of many machines to vary the amount of contained blood as the flow rate through them alters. This attribute may otherwise require quite elaborate calibration if its effect is to be set aside.

Such a system implies that the central venous pressure accurately reflects the blood volume of the patient and it is thus unnecessary to invoke complex methods of measurement of this quantity. Certainly, weighing the patient is as often as misleading as it can be useful.

At the end of perfusion after the venous catheters have been removed, the central venous pressure should be identical with that found before the tubes were inserted. If it is lower then the blood volume is also lower than normal and an arterial transfusion from the machine is needed. In normal circumstances a low venous pressure will be accompanied by a low arterial pressure and both should rise together to normal levels as transfusion proceeds. It is only when a higher than normal venous pressure is found that difficulty may arise. A higher venous pressure accompanying a high or normal arterial pressure, together with a full or distended heart, is easily explained. Venesection, either deliberately, or by virtue of continuing blood loss, will rapidly correct this. However, it is when a higher than normal venous pressure accompanies a low arterial pressure that judgment may be necessary in setting the blood volume at its optimum. Such a set of circumstances inevitably indicates a degree of heart failure and may require that the venous pressure be raised quite markedly to maintain a useful cardiac output. A good example is to be found in the transient tricuspid valve insufficiency which occasionally accompanies the repair of a ventricular septal defect. Traction of the chordae tendineae, displacement of a papillary muscle, or even the presence of the ventricular wound itself may induce a marked reflux of blood from the right ventricle into the right atrium which, in turn, is accompanied by a rise in central venous pressure. To attempt to return to the venous pressure existing before the intracardiac manoeuvre would, of course, be an error in judgment and result in a reduced blood volume, a reduced cardiac output and, inevitably, a low arterial pressure. Treatment of the latter by the administration of vasopressors could only compound the error. An optimum central venous pressure exists in these circumstances and, indeed, where other types of heart failure occur, and it is essential by patient adjustment of the blood volume to determine this. Gross overloading of the

circulation is not likely to be any better tolerated than gross oligaemia, but fear of the former should never allow the latter which must restrict greatly the ability of the damaged heart to maiotain a useful output.

Thus, it may be confidently stated that measurement of the central venous pressure is a reliable guide to the blood volume of patients undergoing heart surgery when an extracorporeal circulation is used. It is suggested that no better method exists even in circumstances when it is of least use, namely, the occasion when heart failure has been created by surgical intervention.

Hypothermia

The most exciting recent development is the re-entry of hypothermia as a manageable asset to extracorporeal circulation. The pioneer work of Gollan (38) has been applied clinically in a number of centres, nowhere more determinedly than by Sealy *et al.* (39). The use of the Brown-Emmons heat exchanger has enabled the body temperature to be manipulated at will with speed and precision and allowed considerable clinical advaoce. Such alterations in temperature need new rules of thumb to guide the perfuser. A good example is to be found io cyanotic heart disease when a substaotial bronchial artery anastomosis may greatly hinder surgery and also allow a substantial proportion of the arterial inflow to go to waste. A reduction of body temperature 28° to 25°C. will enable the arbitrary figure of 2.4 l/min/sq m to be completely effective in providing a full perfusion notwithstanding the extracardiac shunts.

A modification of the technique of Sealy and his colleagues which does not involve an oxygenator, is that of Drew *et al.* (40, 40a), who have made use of the natural lung *in situ*. Two pumps carry the circulation from the right and left heart through a heat exchanger until the body temperature is reduced to below 15°C., at which time the circulations are discontinued. Surgery proceeds in cadaveric conditions with little need for intracardiac suction or even the presence of the cannulating tubes which occupy the right and left atria, the pulmonary artery, and femoral artery. When complete, the extracorporeal circulations are resumed and the cooling process reversed. In practice, good results are obtained by this method in spite of cessation of all circulation for protracted periods, sometimes for as long as an hour. Inherently simple in equipment and sparing of blood, it is a most interesting field and one which may alter our present concepts.

Hypothermia is playing a part in a more regional way and is being used as a method of arresting the heart beat. Gott *et al.* (41) and Shumway *et al.* (42) report its use as a local perfusion of the myocardium to protect the heart itself from the anoxia that attends prolonged ischaemia. They claim a more viable and effective return of contraction than is possible with any other method. Björk in Uppsala (43) reduces the body temperature to low temperatures, then isolates the heart by aortic clamping and immediately begins rewarming the body. The heart itself is kept cold by ice packs. In this manner, he was able to work on the aortic valve for a period of 90 min. with

full recovery. Such a method reduces the rather time-consuming periods required for cooling and rewarming large patients.

Surgery of the aortic valve demands long periods of cardiac ischaemia, and hypothermia either general or local must be considered to be essential. Alternatives such as elective cardiac arrest by potassium citrate or acetylcholine cannot protect the heart for long periods and have indeed been determinedly challenged by Waldhausen *et al.* (44), who maintain that these substances are inherently dangerous. They advocate short periods of aortic clamping as an alternative when this is practicable. This is undoubtedly an effective method and allows an important degree of control over heart block provided the heart beat is never allowed to deteriorate. Electrocardiographic monitoring will aid the placing of stitches only if the rhythm is preserved by adjusting the periods of ischaemia to suit any given conditions.

Dodrill & Takagi (45) have amplified the period of elective arrest by potassium citrate in an ingenious manner. They have supplied ATP in the cardioplegic mixture and hence allowed a much longer time of anoxic metabolism. However, it seems likely that alternatives to the chemical methods of controlling the heartbeat will prevent full use of their discovery.

LITERATURE CITED

1. Gibbon, J. H. (Personal communication, 1953)
2. Le Gallois, L. L., quoted by Belt, A. E., Smith, H. P., and Whipple, G. H., *Am. J. Physiol.*, 52, 101 (1920)
3. Brown-Séquard, C-E., *J. de a Physiol. de l'Homme et des Animaux* 1, 95 (1858)
4. Ludwig, C. F., *Die Physiologischen Leistungen des Blutdrucks* (S. Hirsel, Leipsig, Germany, 1865)
5. Schröder, W., *Arch. exptl. Pathols. Pharmakol. Naunyn-Schmiedeberg's*, 15, 364 (1882)
6. Jacobj, C., *Arch. exptl. Pathol. Pharmakol., Naunyn-Schmiedeberg's*, 26, 388 (1890)
7. Brodie, T. G., *J. Physiol. (London)*, 29, 266 (1903)
8. Frey, M., and Gruber, M., *Arch. Anat. u. Physiol. Anat. Abt.*, 9, 519 (1885)
9. Brukhonenko, S., *J. physiol. et pathol. gén.*, 27, 257 (1929)
10. Björk, V. O., *Acta Chir. Scand.*, 96, Suppl. 137 (1948)
11. Miller, B. J., Gibbon, J. H., Jr., and Gibbon, M. H., *Ann. Surg.*, 134, 694 (1951)
12. Dennis, C., Spreng, D. S., Jr., Nelson, G. E., Karlson, K. E., Nelson, R. M., Thomas, J. V., Eder, W. P., and Varco, R. L., *Ann. Surg.*, 134, 709 (1951)
13. Helmsworth, J. A., Clark, L. C.. Jr., Kaplan, S., Sherman, R. T., and Largen, T., *J. Thoracic Surg.*, 24, 117 (1952) and *J. Am. Med. Assoc.*, 150, 451 (1952)
14. Melrose, D. G., Bassett, J. W., Beaconsfield, P., Graber, 1. G., and Shackman, R., *Brit. Med. J.*, II, 57 (1953)
15. Dodrill, F. D., Gerisch, R. A., and Johnson, A., *J. Thoracic Surg.*, 26, 584 (1953)
16. Warden, H. E., Cohen, M., Read, R. C., and Lillehei, C. W., *J. Thoracic Surg.*, 28, 331 (1954)
17. Clowes, G. H. A., Jr., Neville, W. E., Hopkins, A., Anzola, J., and Simeone, F. A., *Surgery*, 36, 557 (1954)
18. Aird, I., Melrose, D. G., Cleland, W. P., and Lynn, R. B., *Brit. med. J.* I, 1284 (1954)
19. Jones, R. E., Donald, D. E., Swan, H. J. C., Harshbarger, H. G., Kirklin, J. W., and Wood, E. H., *Proc. Staff Meetings Mayo Clinic*, 30, 105 (1955)
20. *Extracorporeal Circulation* (Charles C Thomas, Publ., Springfield, Ill., 1957)
21. Hall, J. E., James, P. A., Lucas, B. G. B., and Waterston, D. J., *Thorax*, 13, 34 (1958)
22. Melrose, D. G., *Brit. J. Anesthesia*, 31, 393 (1959)
23. Miller, D. R., and Albritten, F. F., Jr., *Ann. Surg.*, 151, 75 (1960)
24. Donald, D. E., Harshbarger, H. G., and Kirklin, J. W., *Ann. Surg.*, 144 223 (1956)
25. DeWall, R. A., Warden, H. E., Read, R. C., Gott, V. L., Ziegler, N. R., Varco, R. L., and Lillehei, C. W., *Surg. Clin. N. Am.*, 36, 1025 (1956)
26. Clowes, G. H. A., Jr., Hopkins, A., and Neville, W. E., *J. Thoracic Surg.*, 32, 630 (1956)
27. Gentsch, T. O., Bopp, R. K., Siegel, J. H., Cev, M., and Glenn, W. W., *Surgery*, 47, 301 (1960)
28. Crescenzi, A. A., Hofstra, P. C., Sze, K. C., Foster, B. H., Claff, C. L., and Cooper, P., *Surg. Forum*, 10, 610 (1960)
29. Thomas, J. A., *Compt. rend.*, 248, 291 (1959)
30. Benvenuto, R., ond Lewis, F. J., *Surgery*, 46, 1099 (1959)
31. Melrose, D. G., Bramson, M. L., Osborn, J. J., and Gerbode, F., *Lancet*, I, 1050 (1958)
32. Bramson, M. L. (Personal communication, 1960)
33. Kay, E. B., Galadja, J. E., Lux, A., and Cross, F. S., *J. Thoracic Surg.*, 36, 268 (1958)
34. Osborn, J. J., Bramson, M. L., and Gerbode, F., *J. Thoracic, Cardiovascular Surg.*, 39, 427–37 (1960)
35. Panico, F. G., and Neptune, W. B., *Surg. Forum*, 10, 605 (1960)
36. Melrose, D. G., *Minerva Cardioangiologica Europea* (In press, 1960)
37. Telivuo, L. J., *Ann. Chir. et Gynaecol. Fenniae*, 48, 31 (1959)
38. Gollan, F., Blos, P., and Shuman, F. C., *Am. J. Physiol.*, 171, 331 (1952)
39. Sealy, W. C., Brown, I. W., Jr., Young, W. G., Smith, W. W., and Lesage, A. M., *Ann. Surg.*, 150, 627 (1959)
40. Drew, C. E., Keen, G., and Benazon, D. B., *Lancet*, I, 745 (1959)

40a. Drew, C. E., and Anderson, I. A., *Lancet* I, 748 (1959)

41. Gott, V. L., Bartlett, M. M., and Johnson, J. A., *High Energy Phosphate Metabolism in the Arrested Heart* (Sci. Sessions of Am. Heart Assoc., 31st Meeting, Oct. 1958)
42. Shumway, N. E., Lower, R. R., and Stofer, R. C., *Surg. Gynecol. Obstet.*, 109, 750 (1959)
43. Björk, V. O. (Personal communication, 1960)
44. Waldhausen, J. A., Braunwald, N. S., Bloodwell, R. D., Cornell, W. P., and Morrow, A. G., *J. Thoracic Surg.*, 39, 799 (1960)
45. Dodrill, F. D., and Takagi, S., *Surgery*, 47, 314 (1960)

HORMONAL INFLUENCES ON RENAL FUNCTION[1,2]

By Laurence G. Wesson, Jr., M. D.
Department of Medicine, New York University School of Medicine, New York, New York

The subject matter of this review has not previously been discussed separately in the *Annual Review of Medicine* but has been included in more general reviews of the kidney such as those of Earle (66) and Lauson (143). An attempt will therefore be made to summarize the results of older studies as well as to cite recent contributions. Hormonal influences on renal function will be considered only in the narrow sense. Although all endocrine systems affect renal function collaterally through more or less devious pathways, only those systems, in which a circulating secreted humoral agent directly affects some element of the kidney, can be discussed usefully.

Adenohypophysis

Removal of the anterior or the entire pituitary gland in dogs is followed by marked decreases in GFR, ERPF, Tm_{PAH}, and Tm_G (67, 116, 208, 235, 236, 238, 239). The maximal tubular transport rate of glucose is particularly depressed, the extent of which can be only partially lessened by administration of thyroid or adrenocortical extracts or adrenocorticotropin. The depression in function can be prevented, or supranormal values can be attained, by the administration of anterior pituitary extracts, particularly those representing concentrates of somatotropin (92, 239). Sulfate transport is also enhanced by the somatotropic fraction (92), and it may be concluded provisionally that this principle is the only secretion of the adenohypophysis with a significant, direct effect on the kidney.

In man, GFR, ERPF, Tm_{PAH} and Tm_G are depressed in panhypopituitarism (38, 113, 151, 152, 209, 231) and increased above normal in acromegaly (93, 118, 152). Gershberg (91) reports a 40 per cent increase in endogenous creatinine clearance during daily administration of 1 to 10 mg. of human somatotropin in two patients with panhypopituitarism, and increases of roughly 60 per cent in two patients with uremia and one patient with cirrhosis and ascites. Tubular phosphate reabsorption (assuming that creatinine clearance reflects a qualitative increase in GFR) also increased.

Adrenal Cortex

Glomerular filtration rate, ERPF, and Tm_{PAH} or Tm_D are moderately

[1] The survey of the literature pertaining to this review was concluded in June, 1960.

[2] The following abbreviations will be used: ADH (antidiuretic hormone); ERPF (effective renal plasma flow); GFR (glomerular filtration rate); PTE (parathyroid extract); Tm_G (tubular maxima glucose); Tm_D (tubular maxima diodrast); Tm_{PAH} (tubular maxima *p*-aminohippurate); Tm_{PO_4} (tubular maxima phosphate).

depressed in adrenocortical deficiency in dogs (90, 237) and man (152, 194, 209, 231), and are enhanced in the adrenoprival dog or man by the administration of deoxycorticosterone, cortisone, or adrenocortical extract. The extent to which these effects are attributable to hypovolemia, hypotension, or vascular hypotonia is unknown but is probably considerable. Administration of cortisone or adrenocorticotropin to normal man is accompanied by little if any increase in Tm_{PAH} or Tm_{G}, although some increase in GFR and ERPF may be observed (6, 37, 63, 86, 117, 120, 121, 136, 174). All of the adrenocortical steroids appear to facilitate renal tubular sodium chloride reabsorption although this effect, as reflected in a decrease in NaCl excretion, may frequently be masked in the case of glucocorticoids by the latter's stimulating effect on GFR. Increased NaCl reabsorption following the administration of corticoids in adrenoprival dogs is particularly marked at relatively low Na excretion rates, and decreases in Na excretion of as much as 0.2 mM/min. may be observed following deoxycorticosterone administration to adrenalectomized dogs (89, 177, 181, 218). At high rates of NaCl excretion such as accompany saline loading, no clear difference between normal and adrenalectomized dogs is evident in NaCl reabsorption (179, 189).

Barger, Berlin & Tulenko (15) and Ganong & Mulrow (88) infused aldosterone into one renal artery of dogs. They observed that a decrease in sodium excretion and increase in potassium excretion became evident in about 30 min. but required several hours to attain maximum effect. Relatively little steroid appeared to be extracted by the organ during a single circulation. August & Nelson (13) observed a similar time course in the decrease in NaCl excretion during aldosterone infusion in man, and an hour was required before the stimulating effect of aldosterone on the sodium transport by the isolated toad bladder was evident [Crabbé (48)]. Davey & Lockett (53) demonstrated that antinatriuresis occurs following perfusion of the isolated cat kidney by aldosterone at a blood concentration of 0.5μg./150 ml. but only if oxytocin is also present. In the absence of oxytocin, sodium excretion increased. The mechanism of this interesting effect remains obscure because of the simultaneous increases in renal hemodynamic functions following addition of either aldosterone or oxytocin to the perfusate. Vander, Wilde & Malvin (229) and Vander *et al.* (228), employing the stop-flow technique, and Nicholson (166), employing retrograde $HgCl_2$ injection or intra-arterial tartrate to inflict damage predominantly on distal and proximal segments, respectively, of the nephron, have concluded that aldosterone and deoxycorticosterone act predominantly on "distal" regions of the nephron.

A field of theoretical interest and of possibly important clinical applications has been opened by observations that certain steroids and related compounds may inhibit adrenocortical stimulation of electrolyte transport. Kagawa (128), Landau *et al.* (138), and Landau & Lugibihl (139) observed that progesterone increased Na excretion or blocked the sodium-retaining

action of deoxycorticosterone or aldosterone in humans and rats. No effect was observed in patients with Addison's disease. Similar effects following testosterone administration were observed in the rat by Kagawa & Jacobs (129), and following synthetic steroidal gamma lactones given to human subjects by Liddle (147) and Playoust & Blackburn (170), and seen in the salt-deficient rat by Singer (192). Vander, Wilde & Malvin (229) report that minimum sodium concentrations attained in "distal" tubular sodium samples during stop-flow experiments in the dog are increased to a degree commensurate with those observed following adrenalectomy. Crabbe (48) has reported that spirolactone blocks the stimulating effect of aldosterone on sodium transport by the isolated toad bladder. Potassium excretion is depressed simultaneously as sodium excretion is increased [del Greco (58)].

At the same time that NaCl reabsorption is stimulated by adrenocortical steroids, potassium and hydrogen ion secretion is stimulated, presumably by facilitating the exchange of these intracellular ions with intratubular sodium ions (13, 15, 63, 88, 181). Ammonia excretion is depressed in Addison's disease (127, 146, 149) and in the adrenoprival dog (106) and rat (31), although a metabolic acidosis is usually present. Although this depressed excretion may be attributed in part to an inadequate intratubular supply of hydrogen ions, it is also evident that the increase in ammonia excretion that is observed during chronic acidosis in normal subjects has failed to occur.

The glucocorticoids and ACTH also affect the renal excretion of urate, phosphate, and proteins. Administration of steroids to both normal and gouty subjects is followed acutely by an increase in urate clearance and a slower decrease in plasma urate concentration (100, 120, 121, 199). Whether the increase in excretion reflects a decrease in reabsorption or increase in secretion of urate is unknown. Steroids cause Tm_{PO_4} to decrease in the dog [Roberts & Randall (178)], and phosphate clearance to increase in man [Ingbar *et al.* (120, 121)]. Anderson & Foster (8) found that Tm_{PO_4} decreased about 30 per cent in three patients with Addison's disease receiving 75 mg. of cortisone daily and decreased by roughly the same amount in two of three normal subjects receiving 100 to 200 mg. Smaller quantities (37.5 mg.) caused no change in Tm in the hypoadrenal subjects. Arison & Stoerk (11) showed that the phosphaturic effects of cortisol in the rat could be largely suppressed by parathyroidectomy, although some phosphaturia could still be elicited by massive quantities of cortisol (100 mg./kg.). The mechanism is unclear since, on the one hand, cortisol failed to potentiate the phosphaturic action of parathyroid extract in parathyroidectomized rats and, on the other hand, lack of significant hypercalcemia (in other studies) does not seem to be compatible with steroid stimulation of parathyroid secretion. Protein excretion frequently may decrease during or immediately following chronic administration of ACTH or glucocorticoids in nephrotic patients, particularly those with "lipid" nephrosis. The decrease in proteinuria is attributable almost entirely to a decrease in filtration of albumin [Lauson *et al.* (144, 145)]. In addition, albumin reabsorption may increase slightly to moderately

in some patients during steroid therapy [Lambert *et al.* (137); Malmendier, Grégoire & Lambert (155)], but the data are not yet sufficient to be conclusive.

Adrenal Medulla

The principal effects of the two secretory products of the adrenal medulla, epinephrine and norepinephrine, are on the renal circulation. In man, for whom the most extensive studies are available, injection of these amines is invariably associated with a decrease in ERPF. The glomerular filtration rate is unchanged or may increase slightly with small quantities, but is decreased moderately when the decrease in renal plasma flow is large. The results of some studies are detailed for convenience in Table I.

In addition to their effect on renal hemodynamics, these amines depress potassium excretion acutely and chronically in humans (64, 165, 171), dogs (27, 28) and rats (74, 95). At the same time, urine acidity may decrease [Schlegel (182)], an effect which is contrary to the usual pattern of an inverse relationship between urinary potassium and acid excretion rates. The mechanisms are unknown and no studies have appeared recently.

Gonads

Gonadal deficiency or the administration of excess testosterone or estradiol has little effect on GFR or ERPF in man (57, 62, 132, 140, 142); or dog (176, 232, 237). No effect on Tm_D was observed following ovariectomy in the dog (237), or on Tm_{PAH} or Tm_D following the administration of estradiol or testosterone in the dog (176), or of testosterone (57, 132, 142) or estradiol (57) in man. An increase in Tm_{PAH} associated with testosterone administration has, however, been reported in the dog in one study (232); and the compensatory increase in function (GFR, Tm_D) following unilateral nephrectomy in man is reportedly enhanced by testosterone (142). No effect of estradiol or testosterone on Tm_G in man has been observed (57, 132).

Estradiol has been found to decrease tubular reabsorption of ascorbate in the dog (185), but no changes in ascorbate excretion attributable to changing ovarian function have been seen during the menstrual cycle in women according to Hauck (108).

Neurohypophysis

The neurohypophysis is represented by a mass of histologically and functionally homogeneous tissue composed of the median eminence, the pituitary stalk, and the pars nervosa linked functionally through the hypothalamico-neurohypophyseal tracts to the supraoptic and paraventricular hypothalamic nuclei and possibly, to a lesser extent, to other hypothalamic nuclei [Fisher, Ingram & Ranson (79)]. Two secretions are recognized: an oxytocic principle, the activity of which can be accounted for by the octapeptide, oxytocin; and a pressor-antidiuretic principle the activity of which can be accounted for by the octapeptide, vasopressin. Although these prin-

TABLE I

EFFECTS OF SYMPATHOMIMETIC AMINES ON RENAL PLASMA FLOW AND GLOMERULAR FILTRATION RATE IN MAN*

Substance and method of administration	Mean per cent change ERPF	Mean per cent change GRF	Reference
Adrenalin {0.5–0.8 mg., i.m.	−44	−24	Barclay, Cooke & Kenney (14)
Adrenalin {0.5–10 μgm./min., i.v.	−44	−15	
Epinephrine {1.0–1.5 mg., half i.m., half s.c.	−45	− 9	Maxwell *et al.* (156)
Epinephrine {3–10 μgm./min., i.v.	−33	−15	
Adrenalin 1 mg. s.c.	−38	− 4	Smith (196)
Adrenalin 1 mg. half i.m., half s.c.	−28	+ 4	Smith *et al.* (197)
Adrenalin 1 mg. s.c.	−45	− 1	Chasis *et al.* (43)
Epinephrine 0.013–0.015 mg./kg. s.c.	−23	− 7	Dupré & Coxon (65)
Epinephrine (USP) } 2–46 μgm./min., i.v.	−15	+ 6	Smythe, Nickel & Bradley (198)
L-epinephrine } rate adjusted to sustain	−22	0	
L-norepinephrine } 25–50 mm. Hg B.P. rise	−28	− 3	
L-norepinephrine } rate adjusted to sustain	−21	− 5	Nickel *et al.* (165)
Ephedrine } 25–50 mm. Hg B.P. rise	+ 6	+ 1	
L-norepinephrine {2–4 μgm./min., i.v.	−20	0	Pullman & McClure (172)
L-norepinephrine {10–40 μgm./min., i.v.	−50	−5 to −20	
L-norepinephrine 20–30 μgm./min., i.v.	−28	− 5	Barnett *et al.* (18)
Epinephrine {100 μgm./hr. i.v.	−21	+ 5	Koza, Kottke & Olson (133)
Epinephrine {300 μgm./hr. i.v.	−30	+ 4	
Epinephrine {100 μgm./hr. i.v.†	−18	+ 1	
Epinephrine {100 μgm./hr. i.v.‡	−23	− 9	
L-norepinephrine 12–40 μgm./min., i.v.	−23	− 3	Werko *et al.* (233)
Epinephrine 14–40 μgm./min., i.v.	−16	+ 2	
Epinephrine 10–18 μgm./min., i.v.	−41	−15	Jacobson, Hammarsten & Heller (124)
Norepinephrine 0.16–0.50 μg./kg./min., i.v.	−40	− 6	Mills, Moyer & Skeleton (158)
Epinephrine**	−15	+ 1	Churchill-Davidson & Wylie (44)
Norepinephrine**	−18	+ 2	
Deoxyephedrine**	+19	+30	

* All studies on normal subjects unless otherwise indicated.

† Hypertensive patients: av. B.P. 189/116.

‡ Sympathectomized patients: av. B.P. 189/114.

** Patients anesthetized with ether or cyclopropane.

ciples probably do not circulate in the blood entirely as the free octapeptides (143a), most studies have been based upon the latter.

Oxytocin has been reported to be chloruretic and kaliuretic in most studies on the rat (16, 35, 36, 50, 84, 123, 134, 169), an effect attributed, at least in part, to increase in GFR (61). Oxytocin in low dosage exerts little chloruretic effect in the dog during water diuresis (10, 34) but may be chloruretic in the oliguric dog particularly following intracarotid injection [Brooks & Pickford (34)]. The latter authors (34), and Ali (7) report that oxytocin in the dog undergoing water diuresis may be associated with substantial increases in ERPF and with lesser increases in GFR; similar changes (but without chloruresis) have been seen by Davey & Lockett (54) in the isolated cat kidney. The hemodynamic effects are not observed during antidiuresis or if small quantities of vasopressin are injected simultaneously (34). A similar enhancement of ERPF and GFR by oxytocin has been reported in the hypophysectomized dog (59). No significant chloruretic effect of oxytocin has been reported in human subjects (35, 41). Kaliuresis following oxytocin injection may be a secondary consequence of increased sodium excretion but this is not clearly established.

The pressor-antidiuretic principle (vasopressin) is chloruretic and kaliuretic when injected in large quantities in the antidiuretic dog (87, 188, 201), and rat (5, 47, 50, 51, 61, 101, 123, 134, 161, 164, 190, 191). Small quantities are chloruretic and kaliuretic in the water-diuresing dog (10, 179, 181, 188, 227) but not clearly so in the rat (123, 161). Thorn (220) reports that small quantities (up to 1.08 and 2.16 milliunits, respectively) of synthetic arginine and lysine vasopressin are not chloruretic in the rat during water diuresis. The chloruretic effect of large quantities in the dog (188) and rat (61) has been attributed, at least in part, to increase in filtration rate, but no increase in GFR has been observed during chloruresis following smaller quantities of vasopressin in the dog under water diuresis (10), and the chloruresis associated with large quantities injected into the circulation of the dog heart-lung-kidney preparation was associated with a decrease in blood flow (and probably also in GFR (20)). A significant chloruresis following large doses of vasopressin (3 units) is reported in the normal cat (240). No evident chloruretic effect from small quantities (0.1 to 2.0 mU.) of vasopressin was observed in the diabetes insipidus dog (104), and a decreased chloruretic response as compared with the normal response is reported in the diabetes insipidus cat (240).

Studies are about evenly divided as to whether vasopressin does (9, 52, 148, 195) or does not (163, 168, 215, 234) exert a small chloruretic effect in man. Assali, Dignam & Longo (12) observed a decrease in GFR, ERPF, and excretion of NaCl following the intravenous injection of 100 mU. of vasopressin or continuous infusion of vasopressin at 100 mU./hr. in pregnant women. Few changes in these functions were observed in normal women, however.

The kaliuresis observed following vasopressin administration in the rat and dog may, as in the case of oxytocin, be secondary to increased sodium excretion.

Clarification of the loci of the antidiuretic action of vasopressin has become nearly complete. Apparently, both the distal convoluted tubule and the collecting ducts are relatively impermeable to water in the absence of the antidiuretic hormone; but, in the presence of sufficient quantities of ADH, permeability increases sufficiently to permit osmotic equilibration across the epithelia of these two segments to become virtually complete. A number of studies have shown that tubular fluid remains isosmotic with adjacent blood and interstitium throughout the proximal segment (98, 230, 242) and into the loop of Henle (33, 97, 98, 141, 243). Somewhere near the end of the loop of Henle and probably in the outer medulla, the tubular fluid becomes hyposmotic (33, 97, 98, 242). In rats with diuresis from water or diabetes insipidus, the fluid remains hypotonic until it enters the bladder as hypotonic urine (33, 98, 242). In the presence of vasopressin or antidiuretic stimuli, however, the tubular fluid becomes isosmotic with cortical blood by the midpoint of the distal convolution (97, 98, 242). The pattern is repeated in the collecting ducts. During water diuresis, collecting duct fluid is hypotonic to the medullary interstitium and usually to cortical blood as well (33, 225) but, in the presence of ADH, fluid and medulla attain equal osmotic concentrations (97, 98, 141, 183, 184, 225, 226, 241, 243). Some interesting unanswered questions are: whether ADH affects the medullary blood flow; whether the permeability responses of distal segment and collecting duct to the hormone are similar; and whether the permeability of these epithelia to solutes, such as urea, is affected by it. With respect to medullary blood flow, studies of Frey (85) and of Thurau *et al.* (221, 222) raise the possibilities that the volume or the character of medullary blood flow may change during both water and osmotic diuresis. With respect to permeability to solutes, studies of Jaenike & Berliner (125) which showed a decrease in the urine/medulla concentration gradient of urea in the presence of vasopressin, suggest that this hormone may increase permeability to urea as well as to water.

Holmes (115) reports changes in *in vitro* renal oxygen consumption by fish kidney (trout) following earlier parenteral injection of 10 mU. of neurohypophyseal peptides. Vasopressin was associated with a moderate (up to 20 per cent) increase in consumption while oxytocin was associated with a marked (50 per cent) decrease in consumption.

PANCREAS

A few studies of the effects of pancreatic hormones on discrete renal functions have been reported. Maximal rate of glucose reabsorption is normal to slightly elevated and titration splay is normal in uncomplicated diabetes mellitus (75, 167, 202). Insulin injection (20 units intravenously) was followed by a rapid decrease in Tm_G of 12 to 17 per cent in both diabetic and normal subjects (75, 76, 157). The effects of insulin on glucose titration splay have not been reported.

Glucagon (0.2 to 0.5 mg. i.v.) had no effect on Tm_G in the dog according to Serratto & Earle (187). Glomerular filtration rate and ERPF are increased acutely following intravenous injection of glucagon (a situation reminiscent

of that observed following intravenous injections of parathyroid extracts) but these functions were not increased during chronic administration. An increased excretion of phosphate and decreased excretion of acid and ammonia following intravenous administration of 15 to 40 μg. of glucagon to healthy subjects was observed by Butturini & Bonomini (39). A similar increase in phosphate excretion as well as an increase in Na, Cl, K, and I are reported following the intravenous injection of 0.2 to 0.3 mg. glucagon in dogs by Staub *et al.* (200). Elrick *et al.* (72) note that a transitory increase in urate excretion may follow glucagon administration in both normal and gouty subjects.

Parathyroid

The phosphaturic and calciuric effects of parathyroid extracts, together with changes in excretion and plasma concentrations of calcium and phosphate following parathyroidectomy, or in the presence of parathyroid adenomas, has long been recognized. Working hypotheses that have dominated thought in this field were those of Thomson & Collip (219) who held that the primary action of parathyroid secretion was to mobilize calcium and phosphate from bone, with the kidneys responding secondarily to altered plasma concentrations; and those of Albright and co-workers (2, 3, 4, 6) who held that the primary effect of parathormone was decreased renal tubular reabsorption of phosphate, with mobilization of bone salts representing a shift in plasma-bone equilibria secondary to renal-induced plasma concentration changes. Little doubt remains that both views are correct. A direct bone effect is demonstrated by the plasma and bone changes following injection of parathyroid extract into nephrectomized rats or dogs (70, 99, 119, 160, 186, 204, 205, 206, 214, 223), and by local solution of bone juxtaposed to parathyroid tissue *in vivo* as shown by Barnicot (19) and Chang (42).

Information concerning the mechanism of parathyroid effects on the kidney and the precise sequence of events following parathyroidectomy or administration of parathyroid extracts, is complicated by probable species differences in response, frequent concomitant changes in glomerular filtration rate and plasma phosphate concentrations, and heterogeneity of parathyroid extracts including possible reversible inactivation during preparation. In the chicken, in which tubular secretion of phosphate occurs, PTE causes either a decrease in phosphate reabsorption or increase in secretion (54). No phosphaturic response to massive quantities of PTE was observed in the normal sheep (150), and plasma phosphate did not decrease during PTE administration or following parathyroidectomy in the rabbit although calcium exhibited the expected changes (45). In the parathyroidectomized dog, Tm_{PO_4} has been variously reported as unchanged (81) or increased (114). Administration of PTE to either normal or parathyroidectomized dogs is not followed by rapid changes in phosphate reabsorption (77, 80, 82, 83, 103, 114, 126). The evidence from some studies suggests that "titration splay" may be decreased in the parathyroidectomized dog (114) and increased by

PTE (83) without significant change in Tm. Others have failed to discover any change in the phosphate titration curve within 6 hr. of PTE administration (126). Twenty-four hours following the subcutaneous injection of PTE, however, Tm is decreased (107). In rats and mice, parathyroidectomy is associated with an immediate decrease in phosphate excretion with slower rise in plasma phosphate concentration, while administration of PTE to the parathyroidectomized rat and mouse is follnwed by a prompt increase in excretion (25, 207, 210 to 213, 224). The prnbable absence of significant filtration rate changes to explain these observations is suggested by the relative constancy of creatinine excretion (207). The glomerular filtration rate usually increases immediately following the intravennus injection of PTE in the dog (103, 114) and, in addition, plasma phosphate may increase acutely in both dogs and sheep (114, 126, 150). Load changes have been considered sufficient, in these authors' studies, to explain much if not most of the early phosphaturesis observed with PTE.

In man, Tm_{PO_4}, as measured during acute phosphate loading, is not significantly increased in hypoparathyroidism (217). The titration splay, reflecting excretion at altered loads less than Tm values, is very small, however (49, 217). The maximal rate of transport is significantly decreased in hyperparathyroidism as indicated by the increases observed following removal of parathyroid adenomata (175, 193), while chronic administration of PTE is associated with decreases in Tm that are roughly proportional to the dosage (112). The phosphaturia so frequently reported following acute, intravenous administration of PTE to normal subjects (2, 3, 4, 6, 9, 71, 159) has been attributed primarily to an increase in the filtered load by Klein & Gow (131) and by Hiatt & Thompson (112); but Cargill & Witham (40), Berthoud, Courvoisier & Zahnd (24), Jacobs & Verbanck (122), Gershberg, Shields & Kove (94), and McCrory *et al.* (153) failed to detect an acute increase in filtration rate or plasma phosphate concentration sufficient to account for their observed increases in phosphate excretion. Dent (60), on the other hand, usually failed to observe a rapid phosphaturic effect from the extracts available to him. In hypoparathyroid subjects, however, all studies are in agreement that acute phosphaturic effects of PTE injection result from a decrease in reabsorption at loads both above and below Tm levels (49, 112). These studies in summary, suggest the following description of the effect of parathyroid hormone and its extracts on renal function: the most prominent effect of the hormone in small quantities is depression in efficiency of phosphate transport at low loads; with increasing blood concentrations, Tm is also depressed. And, as implied by the observation that reabsorption may decrease or even vanish during prolonged, heavy phosphate loading in normal but not in hypoparathyroid subjects (96, 216), reabsorption may be completely suppressed at prolonged high blood concentrations; onset of the effect of the hormone (or extract) is gradual, being somewhat more rapid in man than in the dog and slower than in the rat. Because of the gradual onset of the hormone's effect, hemodynamic contributions to excretion may be

excretion following triiodothyronine administration (1 mg.) in both normal and parathyroidectomized dogs. Although they interpreted this result as indicating decreased reabsorption of phosphate, their data indicate that both creatinine clearance and plasma phosphate concentration tended to increase following injection of the hormone.

Thyroid influences urinary dilution and the reabsorption of water but the mechanisms have not been entirely established. Thyroidectomy or the administration of thyroid in the normal dog has little effect upon water turnover [Mahoney & Sheehan (154)], although large amounts of thyroid may increase water turnover slightly to moderately (105). In the diabetes insipidus dog and cat, polyuria is greatly enhanced by thyroid administration and is greatly reduced or may subside entirely following thyroidectomy (26, 78, 105, 154, 173). The effects of thyroid administration cannot be duplicated by injection of anterior pituitary extract (17, 111). Polyuria is reported to decrease following thyroidectomy in patients with diabetes insipidus (30, 110), although response to water loading in patients with myxedema, while less than normal, is not depressed greatly according to Bleifer *et al.* (29), and the hypothyroid rat may excrete a water load more rapidly than normal (203). Although much, if not most, of the polyuric effects of thyroid in diabetes insipidus may be explained as the result of changes in filtration rate and solute excretion, the possibility remains that thyroid depresses the sensitivity of the tubular epithelium to small amounts of vasopressin, a view supported by the observation of Heinbecker, White & Rolf (111) that the maximal antipolyuric effects of thyroidectomy in the diabetes insipidus dog require the presence of vestiges of neurohypophyseal tissue.

Impairment of maximal urinary concentration may occur occasionally in hyperthyroidism in association with hypercalcemia (73, 180).

LITERATURE CITED

1. Aas, K., and Blegen, E., *Scand. J. Lab. and Clin. Invest.*, 1, 22 (1949)
2. Albright, F., Bauer, W., Cockrill, J. R., and Ellsworth, R., *J. Clin. Invest.*, 9, 659 (1931)
3. Albright, F., and Ellsworth, R., *J. Clin. Invest.*, 7, 183 (1929)
4. Albright, F., and Reifenstein, E. C., Jr., *The Parathyroid Glands and Metabolic Bone Disease. Selected Studies* (Williams & Wilkins Co., Baltimore, Md., 1948)
5. Alexander, C. S., *Am. J. Physiol.*, 197, 173 (1959)
6. Alexander, J. D., Pellegrino, E. D., Farber, S. J., and Earle, D. P., *Endocrinology*, 49, 136 (1951)
7. Ali, M. N., *Brit. J. Pharmacol.*, 13, 131 (1958)
8. Anderson, J., and Foster, J. B., *Clin. Sci.*, 18, 437 (1959)
9. Anderson, J. A., and Murlin, W. R., *J. Pediat.*, 21, 326 (1942)
10. Anslow, W. P., Jr., and Wesson, L. G., Jr., *Am. J. Physiol.*, 182, 561 (1955)
11. Arison, R., and Stoerk, H. C., *Federation Proc.*, 19, 159 (1960)
12. Assali, N. S., Dignam, W. J., and Longo, L., *J. Clin. Endocrinol. and Metabolism*, 20, 581 (1960)
13. August, J. T., and Nelson, D. H., *Clin. Research*, 7, 274 (1959)
14. Barclay, J. A., Cooke, W. T., and Kenney, R. A., *Am. J. Physiol.*, 151, 621 (1947)
15. Barger, A. C., Berlin, R. D., and Tulenko, J. F., *Endocrinology*, 62, 804 (1958)
16. Barnafi, L., Rosas, R., de la Lastra, M., and Croxatto, H., *Am. J. Physiol.*, 198, 255 (1960)
17. Barnes, B. O., Regan, J. F., and

prominent during the first hour following injection of PTE but become progressively less significant thereafter.

Several studies indicate a parathyroid effect on renal tubular reabsorption of calcium. Calcium excretion increased transitorily immediately following parathyroidectomy in rats and mice (212, 213) and Bernsteio *et al.* (22) report a decrease in calcium clearance following the administration of PTE in human subjects and an increase io clearance io hypoparathyroidism provided measuremeots are made at constant plasma calcium concentratioos. No clear chaoges in calcium Tm as related to parathyroid function could be discovered by the latter group, suggesting that the priocipal effect of parathyroid hormooe is enhancement of calcium reabsorption at loads less thao the tubular maximum. Laake (135) observed normal calcium clearances io patients with hyperparathyroidism but his data do not permit the conclusioo to be drawn that kinetics of tubular calcium transport are unaltered.

Further advaoces in the study of parathyroid control of renal fuoctioo must, for the most part, await purification and precise chemical and biological characterization of the active principles. Polypeptide fractions of iocreasing activity (up to 7800 USP units/mg.N) have been prepared (162), utilizing chromatographic fractionation. In general, the principal fraction contains both phosphaturic and calcemic activity, and both activities appear to be concentrated equally (162). Bernstein *et al.* (23) report chromatographic separation of three polypeptide fractions: one with calcemic, a second with phosphaturic, and a third with both activities, lending support to the views of earlier workers (55, 102, 130, 205) that the calcium and phosphate effects were separable, and to the surmise of clinical researchers that the ratio of calcium to phosphate activity of commercial PTE has been varying from year to year (60, 159).

Thyroid

The glomerular filtration rate and ERPF are decreased to about half of the expected euthyroid values in myxedema (46, 56, 113, 152, 244). In hyperthyroidism, however, these functions are no more than slightly elevated, and insufficient studies have been reported to determine whether this is significant (1, 32, 46, 113). The filtration fractioo (GFR/ERPF) is not affected by changes in thyroid function. Maximal transport rate of diodrast and Tm_{PAH} are decreased in myxedema (113) and increased in hyperthyroidism (32, 46, 113).

The effects of thyroid function changes, roughly comparable to those observed in human subjects, are observed in the dog. Thyroidectomy is associated with a decrease in ERPF and Tm_D, but with little change in GFR (237). Administration of variable but generally large doses of thyroxine or desiccated thyroid is associated with significant aod frequently large increases in ERPF and Tm_D and also in GFR (68, 105, 109). Maximal rate of glucose reabsorption may increase during thyroid administration by 50 per cent or more (68, 105). Beisel *et al.* (21) report an acute increase in phosphate

excretion following triiodothyronine administration (1 mg.) in both normal and parathyroidectomized dogs. Although they interpreted this result as indicating decreased reabsorption of phosphate, their data indicate that both creatinine clearance and plasma phosphate concentration tended to increase following injection of the hormone.

Thyroid influences urinary dilution and the reabsorption of water but the mechanisms have not been entirely established. Thyroidectomy or the administration of thyroid in the normal dog has little effect upon water turnover [Mahoney & Sheehan (154)], although large amounts of thyroid may increase water turnover slightly to moderately (105). In the diabetes insipidus dog and cat, polyuria is greatly enhanced by thyroid administration and is greatly reduced or may subside entirely following thyroidectomy (26, 78, 105, 154, 173). The effects of thyroid administration cannot be duplicated by injection of anterior pituitary extract (17, 111). Polyuria is reported to decrease following thyroidectomy in patients with diabetes insipidus (30, 110), although response to water loading in patients with myxedema, while less than normal, is not depressed greatly according to Bleifer *et al.* (29), and the hypothyroid rat may excrete a water load more rapidly than normal (203). Although much, if not most, of the polyuric effects of thyroid in diabetes insipidus may be explained as the result of changes in filtration rate and solute excretion, the possibility remains that thyroid depresses the sensitivity of the tubular epithelium to small amounts of vasopressin, a view supported by the observation of Heinbecker, White & Rolf (111) that the maximal antipolyuric effects of thyroidectomy in the diabetes insipidus dog require the presence of vestiges of neurohypophyseal tissue.

Impairment of maximal urinary concentration may occur occasionally in hyperthyroidism in association with hypercalcemia (73, 180).

LITERATURE CITED

1. Aas, K., and Blegen, E., *Scand. J. Lab. and Clin. Invest.*, 1, 22 (1949)
2. Albright, F., Bauer, W., Cockrill, J. R., and Ellsworth, R., *J. Clin. Invest.*, 9, 659 (1931)
3. Albright, F., and Ellsworth, R., *J. Clin. Invest.*, 7, 183 (1929)
4. Albright, F., and Reifenstein, E. C., Jr., *The Parathyroid Glands and Metabolic Bone Disease. Selected Studies* (Williams & Wilkins Co., Baltimore, Md., 1948)
5. Alexander, C. S., *Am. J. Physiol.*, 197, 173 (1959)
6. Alexander, J. D., Pellegrino, E. D., Farber, S. J., and Earle, D. P., *Endocrinology*, 49, 136 (1951)
7. Ali, M. N., *Brit. J. Pharmacol.*, 13, 131 (1958)
8. Anderson, J., and Foster, J. B., *Clin. Sci.*, 18, 437 (1959)
9. Anderson, J. A., and Murlin, W. R., *J. Pediat.*, 21, 326 (1942)
10. Anslow, W. P., Jr., and Wesson, L. G., Jr., *Am. J. Physiol.*, 182, 561 (1955)
11. Arison, R., and Stoerk, H. C., *Federation Proc.*, 19, 159 (1960)
12. Assali, N. S., Dignam, W. J., and Longo, L., *J. Clin. Endocrinol. and Metabolism*, 20, 581 (1960)
13. August, J. T., and Nelson, D. H., *Clin. Research*, 7, 274 (1959)
14. Barclay, J. A., Cooke, W. T., and Kenney, R. A., *Am. J. Physiol.*, 151, 621 (1947)
15. Barger, A. C., Berlin, R. D., and Tulenko, J. F., *Endocrinology*, 62, 804 (1958)
16. Barnafi, L., Rosas, R., de la Lastra, M., and Croxatto, H., *Am. J. Physiol.*, 198, 255 (1960)
17. Barnes, B. O., Regan, J. F., and

Bueno, J. G., *Am. J. Physiol.*, 105, 559 (1933)

18. Barnett, A. J., Blacket, R. B., Depoorter, A. E., Sanderson, P. H., and Wilson, G. M., *Clin. Sci.*, 9, 151 (1950)
19. Barnicot, N. A., *J. Anat.*, 82, 233 (1948)
20. Bayliss, L. E., and Fee, A. R., *J. Physiol.*, 69, 135 (1930)
21. Beisel, W. R., Zerzan, C. J., Jr., Rubini, M. E., and Blythe, W. B., *Am. J. Physiol.*, 195, 357 (1958)
22. Bernstein, D., Kleeman, C. R., Dowling, J. T., and Maxwell, M. H., *Clin. Research*, 7, 246 (1959)
23. Bernstein, D., Kleeman, C. R., Dowling, J. T., and Maxwell, M. H., *Clin. Research*, 8, 139 (1960)
24. Berthoud, E., Courvoisier, B., and Zahnd, G., *Helv. Med. Acta*, 24, 524 (1957)
25. Beutner, E. H., and Munson, P. L., *Endocrinology*, 66, 610 (1960)
26. Biggart, J. H., and Alexander, G. L., *J. Pathol. Bacteriol*, 48, 405 (1939)
27. Blake, W. D., *Am. J. Physiol.*, 181, 417 (1955)
28. Blake, W. D., and Davidson, D. G., *Am. J. Physiol.*, 181, 423 (1955)
29. Bleifer, K. H., Belsky, J. L., Saxon, L., and Papper, S., *J. Clin. Endocrinol. Metabolism*, 20, 409 (1960)
30. Blotner, H., and Cutler, E. C., *J. Am. Med. Assoc.*, 116, 2739 (1941)
31. Boris, A., and Gordon, E. S., *Endocrinology*, 66, 630 (1960)
32. Bradley, S. E., *The Thyroid*, 546 (Hoeber-Harper, New York, N. Y., 1955)
33. Bray, G. A., *Federation Proc.*, 19, 366 (1960)
34. Brooks, F. P., and Pickford, M., *J. Physiol. (London)*, 142, 468 (1958)
35. Brunner, H., Kuschinsky, G., Münchow, O., and Peters, G., *Arch. exptl. Pathol. Pharmakol., Naunyn-Schmiedeberg's*, 230, 80 (1957)
36. Brunner, H., Kuschinsky, G., and Peters, G., *Arch. exptl. Pathol. Pharmakol., Naunyn-Schmiedeberg's*, 228, 457 (1956)
37. Burnett, C. H., *Conf. on Renal Function, Trans.*, 106 (Josiah Macy, Jr., Foundation, New York, N. Y., 1950)
38. Burston, R. A., and Garrod, O., *Clin. Sci.*, 11, 129 (1952)
39. Butturini, U., and Bonomini, V., *Helv. Med. Acta*, 25, 617 (1958)
40. Cargill, W. H., and Witham, A. C., *Federation Proc.*, 8, 21 (1949)
41. Chalmers, T. M., Lewis, A. A. G., and Pawan, G. L. S., *J. Physiol. (London)*, 112, 238 (1951)
42. Chang, H.-Y., *Anat. Record*, 111, 23 (1951)
43. Chasis, H., Ranges, H. A., Goldring, W., and Smith, H. W., *J. Clin. Invest.*, 17, 683 (1938)
44. Churchill-Davidson, H. C., and Wylie, W. D., *Lancet*, II, 803 (1951)
45. Collip, J. B., *Am. J. Physiol.*, 76, 219 (1926)
46. Corcoran, A. C., and Page, I. H., *J. Clin. Endocrinol.*, 7, 801 (1947)
47. Corey, E. L., and Britton, S. W., *Am. J. Physiol.*, 133, 511 (1941)
48. Crabbé, J., *Clin. Research*, 8, 227 (1960)
49. Crawford, J. D., Osborne, M. M., Jr., Talbot, N. B., Terry, M. L., and Morrill, M. F., *J. Clin. Invest.*, 29, 1448 (1950)
50. Croxatto, H., Rosas, R., and Barnafi, L., *Acta Physiol. Latinoam.*, 6, 147 (1956)
51. Croxatto, H., and Zamorano, B., *Acta Physiol. Latinoam.* 7-8, 33 (1957-58)
52. Crutchfield, A. J., Jr., and Wood, J. E., Jr., *Ann. Internal Med.*, 28, 28 (1948)
53. Davey, M. J., and Lockett, M. F., *J. Physiol. (London)*, 152, 206 (1960)
54. Davidson, D. G., and Levinsky, N., *Federation Proc.*, 16, 28 (1957)
55. Davies, B. M. A., and Gordon, A. H., *Nature*, 171, 1122 (1953)
56. Davies, C. E., MacKinnon, J., and Platts, M. M., *Brit. Med. J.*, II, 595 (1952)
57. Dean, A. L., Abels, J. C., and Taylor, H. C., *J. Urol.*, 53, 647 (1945)
58. del Greco, F., *Clin. Research*, 8, 227 (1960)
59. Demunbrun, T. W., Keller, A. D., Levkoff, A. H., and Purser, R. M., Jr., *Am. J. Physiol.*, 179, 429 (1954)
60. Dent, C. E., *Proc. Roy. Soc. (London)*, 46, 291 (1953)
61. Dicker, S. E., and Heller, H., *J. Physiol. (London)*, 104, 353 (1946)
62. Dignam, W. S., Voskian, J., and Assali, N. S., *J. Clin. Endocrinol. Metabolism*, 16, 1032 (1956)
63. Dingman, J. F., Finkenstaedt, J. T., Laidlaw, J. C., Renold, A. E., Jenkins, D., Merrill, J. P., and Thorn, G. W., *Metabolism*, 7, 608 (1958)
64. Duncan, L. E., Jr., Solomon, D. H., Nicholes, M. P., and Rosenberg, E., *J. Clin. Invest.*, 30, 908 (1951)

65. Dupré, J., and Coxon, R. V., *Quart. J. Exptl. Physiol.*, 43, 74 (1958)
66. Earle, D. P., *Ann. Rev. Med.*, 8, 133 (1957)
67. Earle, D. P., Farber, S. J., de Bodo, R. C., Kurtz, M., and Sinkoff, M. W., *Am. J. Physiol.*, 173, 189 (1953)
68. Eiler, J. J., Althausen, T. L., and Stockholm, M., *Am. J. Physiol.*, 140, 699 (1944)
69. Ellsworth, R., *J. Clin. Invest.*, 11, 1011 (1932)
70. Ellsworth, R., and Futcher, P. H., *Bull. Johns Hopkins Hosp.*, 57, 91 (1935)
71. Ellsworth, R., and Howard, J. E., *Bull. Johns Hopkins Hosp.*, 55, 296 (1934)
72. Elrick, H., Whipple, N., Arai, Y., and Hlad, C. J., Jr., *J. Clin. Endocrinol. Metabolism*, 19, 1274 (1959)
73. Epstein, F. H., Freedman, L. R., and Levitin, H., *New Engl. J. Med.*, 258, 782 (1958)
74. Eversole, W. J., Giere, F. A., and Rock, M. H., *Am. J. Physiol.*, 170, 24 (1952)
75. Farber, S. J., Berger, E. Y., and Earle, D. P., *J. Clin. Invest.*, 30, 125 (1951)
76. Farber, S. J., Conan, N. J., Jr., and Earle, D. P., Jr., *Am. J. Physiol.*, 155, 436 (1948)
77. Fay, M., Behrmann, V. G., and Buck, D. M., *Am. J. Physiol.*, 136, 716 (1942)
78. Fisher, C., and Ingram, W. R., *Arch. Internal Med.*, 58, 117 (1936)
79. Fisher, C., Ingram, W. R., and Ranson, S. W., *Diabetes Insipidus and the Neuro-hormonal Control of Water Balance: A Contribution to the Structure and Function of the Hypothalamico-hypophyseal System.*, (Edwards Bros., Ann Arbor, Mich., 1938)
80. Foulks, J. G., *Can. J. Biochem. Physiol.*, 33, 638 (1955)
81. Foulks, J. G., and Perry, F. A., *Am. J. Physiol.*, 196, 554 (1959)
82. Foulks, J. G., and Perry, F. A., *Am. J. Physiol.*, 196, 561 (1959)
83. Foulks, J. G., and Perry, F. A., *Am. J. Physiol.*, 196, 567 (1959)
84. Fraser, A. M., *J. Pharmacol. Exptl. Therap.*, 60, 89 (1937)
85. Frey, E., *Arch. exptl. Pathol. Pharmakol.*, 182, 633 (1936)
86. Froesch, E. R., Winegrad, A. I., Renold, A. E., and Thorn, G. W., *J. Clin. Invest.*, 37, 524 (1958)
87. Fromherz, K., *Arch. exptl. Pathol. Pharmakol.*, 100, 1 (1923)
88. Ganong, W. F., and Mulrow, P. J., *Am. J. Physiol.*, 195, 337 (1958)
89. Garrod, O., Davies, S. A., and Cahill, G., Jr., *J. Clin. Invest.*, 34, 761 (1955)
90. Gaudino, M., and Levitt, M. F., *J. Clin. Invest.*, 28, 1487 (1949)
91. Gershberg, H., *J. Clin. Endocrinol. Metabolism*, 20, 1107 (1960)
92. Gershberg, H., and Gasch, J., *Proc. Soc. Exptl. Biol. Med.*, 91, 46 (1956)
93. Gershberg, H., Heinemann, H. O., and Stumpf, H. H., *J. Clin., Endocrinol. Metabolism*, 17, 377 (1957)
94. Gershberg, H., Shields, D. R., and Kove, S. S., *J. Clin., Endocrinol. Metabolism*, 19, 681 (1959)
95. Giere, F. A., *Endocrinology*, 55, 448 (1954)
96. Goldman, R., and Bassett, S. H., *J. Clin. Endocrinol. Metabolism*, 18, 981 (1958)
97. Gottschalk, C. W., and Mylle, M., *Science*, 128, 594 (1958)
98. Gottschalk, C. W., and Mylle, M., *Am. J. Physiol.*, 196, 927 (1959)
99. Grollman, A., *Endocrinology*, 55, 166 (1954)
100. Gutman, A. B., and Yü, T. F., *Am. J. Med.*, 9, 24 (1950)
101. Ham, G. C., *Proc. Soc. Exptl. Biol. Med.*, 53, 210 (1943)
102. Handler, P., and Cohn, D. V., *Am. J. Physiol.*, 169, 188 (1952)
103. Handler, P., Cohn, D. V., and De Maria, W. J. A., *Am. J. Physiol.*, 165, 434 (1951)
104. Hare, R. S., Hare, K., and Phillips, D. M., *Am. J. Physiol.*, 140, 334 (1943)
105. Hare, K., Phillips, D. M., Bradshaw, J., Chambers, G., and Hare, R. S., *Am. J. Physiol.*, 141, 187 (1944)
106. Harris, F. D., Hartmann, A. F., Jr., Rolf, D., and White, H. L., *Am. J. Physiol.*, 168, 20 (1952)
107. Harrison, H. E., and Harrison, H. C., *J. Clin. Invest.*, 20, 47 (1941)
108. Hauck, H. M., *J. Nutrition*, 33, 511 (1947)
109. Heinbecker, P., Rolf, D., and White, H. L., *Am. J. Physiol.*, 139, 543 (1943)
110. Heinbecker, P., and White, H. L., *Ann. Surg.*, 110, 1037 (1939)
111. Heinbecker, P., White, H. L., and Rolf, D., *Endocrinology*, 40, 104 (1946)
112. Hiatt, H. H., and Thompson, D. D., *J. Clin. Invest.*, 36, 557 (1957)

Bueno, J. G., *Am. J. Physiol.*, 105, 559 (1933)
18. Barnett, A. J., Blacket, R. B., Depoorter, A. E., Sanderson, P. H., and Wilson, G. M., *Clin. Sci.*, 9, 151 (1950)
19. Barnicot, N. A., *J. Anat.*, 82, 233 (1948)
20. Bayliss, L. E., and Fee, A. R., *J. Physiol.*, 69, 135 (1930)
21. Beisel, W. R., Zerzan, C. J., Jr., Rubini, M. E., and Blythe, W. B., *Am. J. Physiol.*, 195, 357 (1958)
22. Bernstein, D., Kleeman, C. R., Dowling, J. T., and Maxwell, M. H., *Clin. Research*, 7, 246 (1959)
23. Bernstein, D., Kleeman, C. R., Dowling, J. T., and Maxwell, M. H., *Clin. Research*, 8, 139 (1960)
24. Berthoud, E., Courvoisier, B., and Zahnd, G., *Helv. Med. Acta*, 24, 524 (1957)
25. Beutner, E. H., and Munson, P. L., *Endocrinology*, 66, 610 (1960)
26. Biggart, J. H., and Alexander, G. L., *J. Pathol. Bacteriol.*, 48, 405 (1939)
27. Blake, W. D., *Am. J. Physiol.*, 181, 417 (1955)
28. Blake, W. D., and Davidson, D. G., *Am. J. Physiol*, 181, 423 (1955)
29. Bleifer, K. H., Belsky, J. L., Saxon, L., and Papper, S., *J. Clin. Endocrinol. Metabolism*, 20, 409 (1960)
30. Blotner, H., and Cutler, E. C., *J. Am. Med. Assoc.*, 116, 2739 (1941)
31. Boris, A., and Gordon, E. S., *Endocrinology*, 66, 630 (1960)
32. Bradley, S. E., *The Thyroid*, 546 (Hoeber-Harper, New York, N. Y., 1955)
33. Bray, G. A., *Federation Proc.*, 19, 366 (1960)
34. Brooks, F. P., and Pickford, M., *J. Physiol. (London)*, 142, 468 (1958)
35. Brunner, H., Kuschinsky, G., Mönchow, O., and Peters, G., *Arch. exptl. Pathol. Pharmakol., Naunyn-Schmiedeberg's*, 230, 80 (1957)
36. Brunner, H., Kuschinsky, G., and Peters, G., *Arch. exptl. Pathol. Pharmakol., Naunyn-Schmiedeberg's*, 228, 457 (1956)
37. Burnett, C. H., *Conf. on Renal Function, Trans.*, 106 (Josiah Macy, Jr., Foundation, New York, N. Y., 1950)
38. Burston, R. A., and Garrod, O., *Clin. Sci.*, 11, 129 (1952)
39. Butturini, U., and Bonomini, V., *Helv. Med. Acta*, 25, 617 (1958)
40. Cargill, W. H., and Witham, A. C., *Federation Proc.*, 8, 21 (1949)
41. Chalmers, T. M., Lewis, A. A. G., and Pawan, G. L. S., *J. Physiol. (London)*, 112, 238 (1951)
42. Chang, H.-Y., *Anat. Record*, 111, 23 (1951)
43. Chasis, H., Ranges, H. A., Goldring, W., and Smith, H. W., *J. Clin. Invest.*, 17, 683 (1938)
44. Churchill-Davidson, H. C., and Wylie, W. D., *Lancet*, II, 803 (1951)
45. Collip, J. B., *Am. J. Physiol.*, 76, 219 (1926)
46. Corcoran, A. C., and Page, I. H., *J. Clin. Endocrinol.*, 7, 801 (1947)
47. Corey, E. L., and Britton, S. W., *Am. J. Physiol.*, 133, 511 (1941)
48. Crabbé, J., *Clin. Research*, 8, 227 (1960)
49. Crawford, J. D., Osborne, M. M., Jr., Talbot, N. B., Terry, M. L., and Morrill, M. F., *J. Clin. Invest.*, 29, 1448 (1950)
50. Croxatto, H., Rosas, R., and Barnafi, L., *Acta Physiol. Latinoam.*, 6, 147 (1956)
51. Croxatto, H., and Zamorano, B., *Acta Physiol. Latinoam.* 7-8, 33 (1957–58)
52. Crutchfield, A. J., Jr., and Wood, J. E., Jr., *Ann. Internal Med.*, 28, 28 (1948)
53. Davey, M. J., and Lockett, M. F., *J. Physiol. (London)*, 152, 206 (1960)
54. Davidson, D. G., and Levinsky, N., *Federation Proc.*, 16, 28 (1957)
55. Davies, B. M. A., and Gordon, A. H., *Nature*, 171, 1122 (1953)
56. Davies, C. E., MacKinnon, J., and Platts, M. M., *Brit. Med. J.*, II, 595 (1952)
57. Dean, A. L., Abels, J. C., and Taylor, H. C., *J. Urol.*, 53, 647 (1945)
58. del Greco, F., *Clin. Research*, 8, 227 (1960)
59. Demunbrun, T. W., Keller, A. D., Levkoff, A. H., and Purser, R. M., Jr., *Am. J. Physiol.*, 179, 429 (1954)
60. Dent, C. E., *Proc. Roy. Soc. (London)*, 46, 291 (1953)
61. Dicker, S. E., and Heller, H., *J. Physiol. (London)*, 104, 353 (1946)
62. Dignam, W. S., Voskian, J., and Assali, N. S., *J. Clin. Endocrinol. Metabolism*, 16, 1032 (1956)
63. Dingman, J. F., Finkenstaedt, J. T., Laidlaw, J. C., Renold, A. E., Jenkins, D., Merrill, J. P., and Thorn, G. W., *Metabolism*, 7, 608 (1958)
64. Duncan, L. E., Jr., Solomon, D. H., Nicholes, M. P., and Rosenberg, E., *J. Clin. Invest.*, 30, 908 (1951)

156. Maxwell, M. H., Gomez, D. M., Fishman, A. P., and Smith, H. W., *J. Pharmacol. Exptl. Therap.*, 109, 276 (1953)
157. Miller, J. H., *Proc. Soc. Exptl. Biol. Med.*, 84, 322(1953)
158. Mills, L. C., Moyer, J. H., and Skelton, J. M., *Am. J. Med. Sci.*, 226, 653 (1953)
159. Milne, M. D., *Clin. Sci.*, 10, 471 (1951)
160. Monahan, E. P., and Freeman, S., *Am. J. Physiol.*, 142, 104 (1944)
161. Morel, F, *Bull. biol. France et Belg.*, Suppl. 34, (1955)
162. Munson, P. L., *Federation Proc.*, 19, 593 (1960)
163. Murphy, R. J. F., and Stead, E. A., Jr., *J. Clin. Invest.*, 30, 1055 (1951)
164. Nelson, E. E., and Woods, G. G., *J. Pharmacol. Exptl. Therap.*, 50, 241 (1934)
165. Nickel, J. F., Smythe, C. M., Papper, E. M., and Bradley, S. E., *J. Clin. Invest.*, 33, 1687 (1954)
166. Nicholson, T. F., *Can. J. Biochem. Physiol.*, 35, 641 (1957)
167. Nielsen, A. L., *Acta Med. Scand.*, 130, 219 (1948)
168. Pasqualini, R. Q., and Etala, E., *Rev. soc. arg. biol.*, 17, 198 (1941)
169. Peters, G., *Arch. exptl. Pathol. Pharmakol.*, 235, 335 (1959)
170. Playoust, M. R., and Blackburn, C. R. B., *Australasian Ann. Med.*, 9, 64 (1960)
171. Pullman, T. N., and McClure, W. W., *J. Lab. Clin. Med.*, 39, 711 (1952)
172. Pullman, T. N., and McClure, W. W., *Circulation*, 9, 600 (1954)
173. Radcliffe, C. E., *Endocrinology*, 32, 415 (1943)
174. Raisz, L. G., McNeely, W. F., Saxon, L., and Rosenbaum, J. D., *J. Clin. Invest.*, 36, 767 (1957)
175. Reynolds, T. B., Lanman, H., and Tupikova, N., *J. Clin. Endocrinol. Metabolism*, 20, 1136 (1960)
176. Richardson, J. A., and Houck, C. R., *Am. J. Physiol.*, 165, 93 (1951)
177. Roberts, K. E., and Pitts, R. F., *Endocrinology*, 50, 51 (1952)
178. Roberts, K. E., and Randall, H. T., *Ann. N. Y. Acad. Sci.*, 61, 306 (1955)
179. Roemmelt, J. C., Sartorius, O. W., and Pitts, R. F., *Am. J. Physiol.*, 159, 124 (1949)
180. Sallin, O., *Acta Endocrinol.*, 29, 425 (1958)
181. Sartorius, O. W., and Roberts, K., *Endocrinology*, 45, 273 (1949)
182. Schlegel, J. U., *Am. J. Physiol.*, 168, 522 (1952)
183. Schmidt-Nielsen, B., and O'Dell, R., *Am. J. Physiol.*, 197, 856 (1959)
184. Schmidt-Nielsen, B., and O'Dell, R., *Federation Proc.*, 19, 366 (1960)
185. Selkurt, E. E., Talbot, L. J., and Houck, C. R., *Am. J. Physiol.*, 140, 260 (1943)
186. Selye, H., *Arch. Pathol.*, 34, 625 (1942)
187. Serratto, M., and Earle, D. P., *Proc. Soc. Exptl. Biol. Med.*, 102, 701 (1959)
188. Shannon, J. A., *J. Exptl. Med.*, 76, 387 (1942)
189. Share, L., and Hall, P. W., III, *Am. J. Physiol.*, 183, 291 (1955)
190. Silvette, H., *Am. J. Physiol.*, 128, 747 (1940)
191. Silvette, H., *Proc. Soc. Exptl. Biol. Med.*, 45, 599 (1940)
192. Singer, B., *Endocrinology*, 65, 512 (1959)
193. Sirota, J. H., *Federation Proc.*, 12, 133 (1953)
194. Skillern, P. G., Corcoran, A. C., and Scherbel, A. L., *J. Clin. Endocrinol. Metabolism*, 16, 171 (1956)
195. Smith, F. M., and MacKay, E. M., *Proc. Soc. Exptl. Biol. Med.*, 34, 116 (1936)
196. Smith, H. W., *Harvey Lectures*, 35, 166 (1939)
197. Smith, H. W., Goldring, W., Chasis, H., Ranges, H. A., and Bradley, S. E., *J. Mt. Sinai Hosp.*, 10, 59 (1943)
198. Smythe, C. M., Nickel, J. F., and Bradley, S. E., *J. Clin. Invest.*, 31, 499 (1952)
199. Sprague, R. G., Power, M. H., Mason, H. L., Albert, A., Mathieson, D. R., Hench, P. S., Kendall, E. C., Slocumb, C. H., and Polley, H. F., *Arch. Internal. Med.*, 85, 199 (1950)
200. Staub, A., Springs, V., Stoll, F., and Elrick, H., *Proc. Soc. Exptl. Biol. Med.*, 94, 57 (1957)
201. Stehle, R. L., and Bourne, W. J., *J. Physiol. (London)*, 60, 229 (1925)
202. Steinitz, K., *J. Clin. Invest.*, 19, 299 (1940)
203. Stephan, F., Jahn, H., and Metz, B., *Compt. rend.*, 248, 1227 (1959)
204. Stewart, G. S., and Bowen, H. F., *Endocrinology*, 48, 568 (1951)
205. Stewart, G. S., and Bowen, H. F., *Endocrinology*, 51, 80 (1952)
206. Stoerk, H. C., *Proc. Soc. Exptl. Biol. Med.*, 54, 50 (1943)
207. Stoerk, H. C., and Silber, R. H., *Lab. Invest.*, 5, 213 (1956)

113. Hlad, C. F., and Bricker, N. S., *J. Clin. Endocrinol. Metabolism*, 14, 1539 (1954)
114. Hogben, C. A., and Bollman, J. L., *Am. J. Physiol.*, 164, 670 (1951)
115. Holmes, W. N., *Acta Endocrinol.*, 33, 428 (1960)
116. Howell, D. S., Davis, J. O., and Laquer, G. L., *Circulation Research*, 3, 264 (1955)
117. Huffman, E. R., Wilson, G. M., Jr., Clark, G. M., and Smyth, C. J., *J. Lab. Clin. Med.*, 47, 747 (1956)
118. Ikkos, D., Ljunggren, H., and Luft, R. *Acta Endocrinol.*, 21, 226 (1956)
119. Ingalls, T. H., Donaldson, G., and Albright, F., *J. Clin. Invest.*, 22, 603 (1943)
120. Ingbar, S. H., Relman, A. S , Burrows, B. A., Kass, E. H., Sisson, J. H., and Burnett, C. H., *J. Clin. Invest.*, 29, 824 (1950)
121. Ingbar, S. H., Kass, E. H., Burnett, C. H., Relman, A. S., Burrows, B. A., and Sisson, J. H., *Proc. Clin. ACTH Conf., 2nd Conf.*, 1, 130 (1951)
122. Jacobs, E., and Verbanck, M., *Acta Med. Scand.*, 145, 143 (1953)
123. Jacobson, H. N., and Kellogg, R. H., *Am. J. Physiol* , 184, 376 (1956)
124. Jacobson, W. E., Hammarsten, J. F., and Heller, B. I., *J. Clin. Invest.*, 30, 1503 (1951)
125. Jaenike, J. R., and Berliner, R. W., *Clin. Research*, 8, 229 (1960)
126. Jahan, I., and Pitts, R. F., *Am. J. Physiol.*, 155, 42 (1948)
127. Jimenez-Diaz, C., *Lancet*, II, 1135 (1936)
128. Kagawa, C. M., *Proc. Soc. Exptl. Biol. Med.*, 99, 705 (1958)
129. Kagawa, C. M., and Jacobs, R. S , Jr., *Proc. Soc. Exptl. Biol. Med.*, 102, 521 (1959)
130. Kenny, A. D., Vine, B. G., and Munson, P. L., *Federation Proc.*, 13, 240 (1954)
131. Klein, R., and Gow, R. C., *J. Clin. Endocrinol. Metabolism*, 13, 271 (1953)
132. Klopp, C., Young, N. F., and Taylor, H. C., Jr., *J. Clin. Invest.*, 24, 189 (1945)
133. Koza, D. W., Kottke, F. J., and Olson, M., *J. Appl. Physiol.*, 3, 610 (1951)
134. Kuschinsky, G., and Bundschuh, H. E., *Arch. Exptl. Pathol. Pharmakol* , 192, 683 (1939)
135. Laake, H., *Acta Med. Scand.*, 165, 71 (1959)
136. Laidlaw, J. C., Dingman, J. F., Arons, W. L., Finkenstaedt, J. T., and Thorn, G. W., *Ann. N. Y. Acad. Sci.*, 61, 315 (1955)
137. Lambert, P. P., Grégoire, F., Malmendier, C., Vanderveiken, F., and Gueritte, G., *Bull. acad. roy. méd. Belg.*, 22, 524 (1957)
138. Landau, R. L., Bergenstal, D. M., Lugibihl, K., and Kascht, M. E., *J. Clin. Endocrinol. Metabolism*, 15, 1194 (1955)
139. Landau, R. L., and Lugibihl, K., *J. Clin. Endocrinol. Metabolism*, 18, 1237 (1958)
140. Langeron, L., Nolf, V., and Liefooghe, J., *J. urol. méd. et chir.*, 57, 293 (1959)
141. Lassiter, E. W., Gottschalk, C. W., and Mylle, M., *Federation Proc.*, 19, 369 (1960)
142. Lattimer, J. K., *J. Urol.*, 48, 778 (1942)
143. Lauson, H. D., *Ann. Rev. Med.*, 9, 125 (1958)
143a. Lauson, H. D., in *Hormones in Human Plasma* (Little, Brown & Co., Boston, Mass., 1960.
144. Lauson, H. D., Forman, C. W., McNamara, H., Mattar, G., and Barnett, H. L., *Am. J. Diseases Children*, 83, 87 (1952)
145. Lauson, H. D., Forman, C. W., McNamara, H., Mattar, G., and Barnett, H. L., *J. Clin. Invest.*, 33, 657 (1954)
146. Levy, M. S., Power, M. H., and Kepler, E. J., *J. Clin. Endocrinol.*, 6, 607 (1946)
147. Liddle, G. W., *Science*, 126, 1016 (1957)
148. Little, J. M., Wallace, S. L., Whatley, E. C., and Anderson, G. A., *Am. J. Physiol.*, 151, 174 (1947)
149. Loeb, R. F., Atchley, D. W., and Stahl, J., *J. Am. Med. Assoc.*, 104, 2149 (1935)
150. Lotz, W. E., Talmage, R. V., and Comar, C. L., *Proc. Soc. Exptl. Biol. Med.*, 85, 292 (1954)
151. Luft, R., and Sjögren, B., *Acta Endocrinol.*, 2, 44 (1949)
152. Luft, R., and Sjögren, B., *Acta Endocrinol.*, 4, 351 (1950)
153. McCrory, W. W., Forman, C. W., McNamara, H., and Barnett, H. L., *J. Clin. Invest.*, 31, 357 (1952)
154. Mahoney, W., and Sheehan, D., *Am. J. Physiol.*, 112, 250 (1935)
155. Malmendier, C., Grégoire, F., and Lambert, P. P., *Rev. franç études clin. et biol.*, 2, 145 (1957)

KIDNEY DISEASE: ACQUIRED TUBULAR DISORDERS

(With Special Reference to Disturbances of Concentration and Dilution and of Acid-Base Regulation)[1,2]

By Arnold S. Relman, M.D. and Norman G. Levinsky, M.D.
Department of Medicine, Boston University School of Medicine, and the Evans Memorial Department of Clinical Research, Massachusetts Memorial Hospitals, Boston, Massachusetts

Many aspects of tubular dysfunction in both acquired and hereditary renal disease have been extensively reviewed within the past five years (1 to 5). Of particular interest has been the rapid development of information about the structural and functional consequences of acquired potassium depletion (5 to 9) and calcium excess (10, 11), which also have been the subjects of recent and comprehensive reviews. This article will therefore concern itself exclusively with two other aspects of acquired tubular disease which, though currently under active investigation, have not received fully adequate attention in recent review articles. We refer to (*a*) acquired disorders of urine concentration and dilution and (*b*) acquired disorders of acid-base regulation. In discussing these topics we have not limited our reference material to any arbitrary interval of time, for such practice tends to impose unreasonable restraints on the logical exposition of ideas. On the other hand, neither have we attempted to review, or even mention, every available article in the modern literature on these two subjects, because this would obviously be impossible within the present limitations of space. We have tried to solve this dilemma by following a middle policy under which attention has been primarily focused on those studies that have contributed to the development of current ideas in the two fields under review. References have been made to case reports and clinical discussions only as they illustrate or illuminate the points under discussion. Many otherwise interesting articles have had to be omitted.

ACQUIRED DISORDERS OF CONCENTRATION AND DILUTION

Two excellent reviews of some physiological and clinical aspects of this subject have recently appeared (12, 13).

Physiology of Concentration and Dilution

The introduction of the countercurrent multiplier principle into renal physiology by Wirz, Hargitay & Kuhn (14, 15) has greatly stimulated in-

[1] The survey of the literature pertaining to this review was concluded in June, 1960.

[2] The following abbreviations will be used: GFR (glomerular filtration rate); RTA (renal tubule acidosis); Tc_{H_2O} (negative free-water clearance when urine is hypertonic); C_{H_2O} (positive free-water clearance when urine is hypotonic).

208. Surtshin, A., Rolf, D., and White, H. L., *Am. J. Physiol.*, 165, 429 (1951)
209. Talbott, J. H., Pecora, L. J., Melville, R. S., and Consolazio, W. V., *J. Clin. Invest.*, 21, 107 (1942)
210. Talmage, R. V. and Kraintz, F. W., *Proc. Soc. Exptl. Biol. Med.*, 85, 416 (1954)
211. Talmage, R. V., and Kraintz, F. W., *Proc. Soc. Exptl. Biol. Med.*, 87, 263 (1954)
212. Talmage, R. V., Kraintz, F. W., and Buchanan, G. D., *Proc. Soc. Exptl. Biol. Med.*, 88, 600 (1955)
213. Talmage, R. V., Kraintz, F. W., and Buchanan, G. D., *Federation Proc.*, 18, 155 (1959)
214. Talmage, R. V., Kraintz, F. W., Frost, R. C., and Kraintz, L., *Endocrinology*, 52, 318 (1953)
215. Tarail, R., and Mateer, F. M., *Federation Proc.*, 10, 135 (1951)
216. Thompson, D. D., and Hiatt, H. H., *J. Clin. Invest.*, 36, 566 (1957)
217. Thompson, D. D., and Hiatt, H. H., *J. Clin. Invest.*, 36, 550 (1957)
218. Thompson, D. D., and Pitts, R. F., *Am. J. Physiol*, 166, 490 (1952)
219. Thomson, D. L., and Collip, J. B., *Physiol. Revs.*, 12, 309 (1932)
220. Thorn, N. A., *Acta Endocrinol.*, 32, 123 (1959)
221. Thurau, K., Deetjen, P., and Kramer, K., *Arch. ges. Physiol.*, 270, 270 (1960)
222. Thurau, K., Kramer, K., Deetjen, P., and Brechtelsbauer, H., *Federation Proc.*, 19, 360 (1960)
223. Toft, R. J., and Talmage, R. V., *Federation Proc.*, 19, 51 (1960)
224. Tweedy, W. R., and Campbell, W. W., *J. Biol. Chem.*, 154, 339 (1944)
225. Ullrich, K. J., Drenckhahn, F. O., and Jarausch, K. H., *Arch. ges. Physiol.*, 261, 62 (1955)
226. Ullrich, K. J., and Jarausch, K. H., *Arch. ges. Physiol.*, 262, 537 (1956)
227. Unna, K., and Wallerskirchen, L., *Arch. exptl. Pathol. Pharmakol.*, 181, 681 (1936)
228. Vander, A. J., Malvin, R. L., Wilde, W. S., Lapides, J., Sullivan, L. P., and McMurray, V. M., *Proc. Soc. Exptl. Biol. Med.*, 99, 323 (1958)
229. Vander, A. J., Wilde, W. S., and Malvin, R. L., *Proc. Soc. Exptl. Biol. Med.*, 103, 525 (1960)
230. Walker, A. M., Bott, P. A., Oliver, J., and MacDowell, M. C., *Am. J. Physiol.*, 134, 580 (1941)
231. Waterhouse, C., and Keutmann, E. H., *J. Clin. Invest.*, 27, 372 (1948)
232. Welsh, C. A., Rosenthal, A., Duncan, M. T., and Taylor, H. C., Jr., *Am. J. Physiol.*, 137, 338 (1942)
233. Werko, L., Bucht, H., Josephson, B., and Ek, J., *Scand. J. Clin. Lab. Invest.*, 3, 255 (1951)
234. White, A. G., Rubin, G., and Leiter, L., *J. Clin. Invest.*, 30, 1287 (1951)
235. White, H. L., Heinbecker, P., and Rolf, D., *Proc. Soc. Exptl. Biol. Med.*, 46, 44 (1941)
236. White, H. L., Heinbecker, P., and Rolf, D., *Am. J. Physiol.*, 136, 564 (1942)
237. White, H. L., Heinbecker, P., and Rolf, D., *Am. J. Physiol.*, 149, 404 (1947)
238. White, H. L., Heinbecker, P., and Rolf, D., *Am. J. Physiol.*, 156, 67 (1949)
239. White, H. L., Heinbecker, P., and Rolf, D., *Am. J. Physiol.*, 157, 47 (1949)
240. Winter, C. A., Ingram, W. R., Gross, E. G., and Sattler, D. G., *Endocrinology*, 28, 535 (1941)
241. Wirz, H., *Helv Physiol. et Pharmacol. Acta*, 11, 20 (1953)
242. Wirz, H., *Helv. Physiol. et Pharmacol. Acta*, 14, 353 (1956)
243. Wirz, H., Hargitay, B., and Kuhn, W., *Helv. Physiol. et Pharmacol. Acta*, 9, 196 (1951)
244. Yount, E., and Little, J. M., *J. Clin. Endocrinol. Metabolism*, 15, 343 (1955)

decreases both maximum urinary osmolality after vasopressin and $T_{C_{H_2O}}$. The mechanism of this effect is not known; it is apparently not caused by changes in glomerular filtration rate (GFR) or solute excretion (28). Patients with the syndrome of compulsive water drinking and occasionally patients with diabetes insipidus may have an impaired ability to concentrate urine when given vasopressin (30), which may be related to this phenomenon.

Maximum urine osmolality after dehydration or vasopressin administration increases slowly during infancy, reaching adult levels only after several months (31). Edelmann, Barnett & Troupkou (32) have recently re-examined the apparent immaturity of the urine-concentration mechanism in infants in the light of the observations that prior diet and water intake condition urine-concentrating ability in normal subjects. They found that diets high in protein content or supplemented with urea enhanced maximum urine osmolality. Diets with low water content had a similar effect. The authors therefore convincingly argue that the inability of the infant to concentrate its urine as well as the adult is not the result of immaturity of renal tubules, but is determined largely by differences in diet and protein metabolism.

In the clinic, concentrating ability is usually measured in terms of the maximum specific gravity of the urine after a variable period of dehydration. Miles, Paton and de Wardener (33) have found that maximum values for urine specific gravity are obtained only after at least 22 hr. of dehydration. In individuals with relatively normal concentrating ability, the administration of a long-acting vasopressin preparation without water restriction results in somewhat lower urine concentrations than does dehydration for 22 hr. or more. The explanation for this difference is not known; it may be related to the effects of overhydration on concentrating ability as discussed above, or to the diminution of urine osmolality which occurs during chronic vasopressin and water administration (34, 35). In patients with impaired ability to concentrate the urine, dehydration and administration of vasopressin without water restriction appear to be equally effective (33).

Chronic Renal Failure

Some degree of impairment of concentrating and diluting ability is invariably a feature of chronic renal failure. Until relatively recently, it was generally assumed that these defects resulted from anatomical or biochemical damage to concentrating and diluting sites in the renal tubule. However, it had been known since 1939, from the experiments of Hayman *et al.* (36), that concentrating ability is reduced in the presumably normal nephrons remaining after subtotal nephrectomy, and this has been confirmed in a variety of ways since then. More recently, physiological studies with solute infusions (37, 38, 39) have demonstrated that concentration and dilution of the urine are limited during solute (osmotic) diuresis in normal kidneys. Hence, the concentrating defect after subtotal nephrectomy can be interpreted as the physiological consequence of the imposition of the normal load of solute on a reduced number of intact nephron units. Since the total solute

vestigation of the concentrating function of the kidney. Micropuncture studies by Wirz (16) and by Gottschalk's group (17), cannulation of individual collecting ducts by Ullrich and associates (18), and analysis of kidney tissue for various solutes (19, 20) have proven particularly useful in determining the sequence of events along the nephron during concentration and dilution of the urine. An outline of present views of these events is as follows: (*a*) Regardless of the presence or absence of antidiuretic hormone, active reabsorption of sodium out of the loop of Henle dilutes the tubular fluid and creates hypertonic interstitial fluid in the medulla. Interstitial hypertonicity is maintained in part because of the solute-trapping function of the countercurrent circulation to the medulla. (*b*) In the absence of antidiuretic hormone, the distal convoluted tubule and collecting ducts are relatively impermeable to water. The hypotonic fluid from the loop is further diluted by sodium reabsorption in the distal segments, and water diuresis ensues. (*c*) When antidiuretic hormone is present, the distal tubule and collecting ducts become permeable to water. The tubular fluid becomes isotonic in the distal tubule and is greatly reduced in volume. In the collecting ducts, this isotonic fluid equilibrates with the hypertonic medullary interstitial fluid, with the final formation of hypertonic urine. The limitations of space preclude any extended discussion of these ideas; a number of recent reviews of this subject are available (15, 21, 22, 23).

Many years ago, Gamble *et al.* (24) showed that urea feeding enhances concentrating ability in the rat. Recently, Epstein and his associates (25) have applied Gamble's idea that there is "an economy of water in renal function referable to urea" to man. They showed that both maximum urine osmolality and maximum negative free-water clearance (Tc_{H_2O}) are impaired in subjects on a low-protein diet. Merony *et al.* (26) have confirmed this effect of dietary protein on maximum urine osmolality. Acute or chronic administration of urea will almost completely reverse or prevent the concentrating defect induced by a low-protein diet in man (25, 27). Berliner and associates have suggested that these results, as well as many experimental data in animals, can be explained by postulating a unique role for urea in the urine-concentrating process in the medulla of the kidney (21, 27). It was proposed that a small fraction of urine urea passively diffuses across the collecting ducts into the medullary interstitial fluid, where it is trapped by the countercurrent blood flow and becomes concentrated nearly to urine levels. The hypertonicity of the interstitium and thus of the final urine is enhanced. The osmotic force of the urine urea is largely balanced by interstitial urea and sodium transport by the loop needs to be sufficient to balance only urine solutes other than urea. Whatever the mechanism of the urea effect, it is evident that antecedent protein intake must be considered in evaluating clinical tests of concentrating ability.

Another factor of importance in evaluating concentrating ability is the subject's prior state of hydration. Epstein and associates (28) and de Wardener & Herxheimer (29) have shown that previous forced water drinking

the production of the concentrating defect in chronic renal failure by a more direct approach. Solute excretion was acutely reduced at least 60 per cent either by lowering plasma urea levels by means of hemodialysis or by acutely lowering GFR with hypotensive agents. Maximum urine concentration was not increased by either maneuver. In the somewhat similar studies of Levitt *et al.* (52), only slight increases in maximum osmolality occurred when GFR was acutely reduced by 50 per cent or more in seven uremic patients. While more data of this kind are obviously needed, these experiments suggest that the presence of a relative solute diuresis may not be sufficient to account for the concentrating defect in chronic renal failure.

Available data on diluting capacity in renal failure are limited to those reported in an abstract by Kleeman, Adams & Maxwell (53). In 21 patients with unspecified chronic azotemic renal disease, minimum urine osmolality was considered to be normal when "corrected" for the rate of solute excretion per nephron. C_{H_2O}/GFR was decreased in ten cases, normal in seven, and increased in four.

Pyelonephritis

In the preceding section on "chronic renal failure" we have accepted for purposes of discussion the tacit assumption that chronic glomerulonephritis, pyelonephritis, nephrosclerosis, etc., all produce equivalent changes in the concentrating and diluting functions of the kidney. Many clinical studies suggest strongly that interference with the concentrating process is more marked and occurs earlier in pyelonephritis than in several other common disorders usually included in "chronic Bright's disease."

In studies of infants and children with acute, non-obstructive urinary tract infections, Winberg (54) found that maximum urine osmolality was significantly decreased in 17 of 22 patients when first measured during the first week after fever subsided, and this often persisted for several weeks. Repeated creatinine clearances were normal in all but two subjects during this time. Kaitz (55) reported that the mean maximum urine/plasma osmolality of 13 pregnant women with asymptomatic bacteriuria was 2.4 ± 0.7 (S.D.), a clear reduction below the value of 3.5 ± 0.3 for 20 pregnant control subjects. Creatinine clearance and PSP excretion were normal in every case; solute diuresis, hypercalcuria, and potassium depletion were not present.

Raaschou (56) studied 20 patients with chronic pyelonephritis, in whom urea clearances were within the normal range. Maximum urine specific gravity was between 1.010 and 1.016 in the pyelonephritic group, compared with values of 1.019 to 1.028 for a control group. Brod (57) compared the relationship between maximum specific gravity and creatinine clearance in groups of patients with chronic pyelonephritis, chronic glomerulonephritis, or nephrosclerosis. He concluded that pyelonephritis affects urine-concentrating ability more severely than either glomerulonephritis or nephrosclerosis over the entire range of filtration rates. There was, however, considerable scatter of individual cases, and statistical validation of the conclusions was

load requiring excretion by the kidney is probably the same in normal individuals and in those with uremic renal failure, each surviving nephron in such patients is subjected to a similar type of solute diuresis. Smith (40), Platt (41), Strauss (42), Welt (43), de Wardener (44) and many other nephrologists now support the view that the impairment of concentration and dilution in chronic azotemic renal disease is mainly to be attributed to this effect. There is little reason to doubt that much of the limitation on concentration and dilution in chronic renal failure is the consequence of this relative osmotic diuresis.

Recently, Bricker and associates (45, 46) have adduced evidence in a variety of experimental renal diseases in the dog that only intact nephrons contribute significantly to the formation of urine. By inducing the experimental disease in only one kidney of each dog, it was possible to study the "intrinsic" function of the diseased kidney nearly unaffected by the elevated plasma urea levels and the other chemical and physiological abnormalities of uremia. Under these circumstances, the maximum osmolality of urine from the diseased kidneys was only slightly less than that from the contralateral controls, and dilution during water diuresis was normal. To "correct" for the number of intact nephrons in each kidney, free-water clearances were factored by filtration rates. Tc_{H_2O}/GFR was only slightly reduced and C_{H_2O}/GFR was actually somewhat increased in the diseased kidneys as compared to their controls. These results were interpreted (47) as strong evidence that the intrinsic concentrating and diluting mechanisms in the nephrons of the diseased kidney are essentially intact, thus lending support to the idea that relative osmotic diuresis accounts for isosthenuria in uremia.

The results of the limited number of observations on Tc_{H_2O}/GFR in human chronic renal failure are not as uniform as those in Bricker's studies. Baldwin *et al.* (48) found that Tc_{H_2O}/GFR was normal in four patients with chronic glomerulonephritis who had filtration rates between 60 and 70 ml. per minute. Of ten others who had filtration rates of less than 60 ml. per minute, Tc_{H_2O} was normal in five and somewhat reduced in the rest. Nine patients with "chronic nephritis" studied by Dorhout Mees (49, 50) all showed marked decreases in Tc_{H_2O}/GFR.

Implicit in all such clinical and experimental studies is the assumption that marked reductions in the absolute value of Tc_{H_2O} do not represent decreased concentrating capacity if they are proportional to falls in GFR. The validity of this assumption is open to serious question. It would be approximately true only if falls in GFR represented cessation of filtration in whole nephron units. If, as would seem a priori more likely, filtration rate per nephron may vary in renal disease, interpretation of the Tc_{H_2O}/GFR ratio would be very difficult. Experiments in dogs (20) indicate that falls in GFR due, in part, to decreased GFR per functioning nephron unit have variable effects on Tc_{H_2O}/GFR.

Franklin, Niall & Merrill (51) have reported in abstract an attempt to determine the relative roles of solute diuresis and intrinsic tubular damage in

Renal Artery Occlusion

Deming (61) and Margolin, Merrill & Harrison (62) have each described a patient with malignant hypertension caused by renal artery occlusion in whom polyuria and polydipsia were early and striking symptoms. Concentrating ability after dehydration or vasopressin was, in each case, markedly impaired in the absence of significant azotemia. Each paper calls attention to a number of cases culled from the literature in which polyuria was a striking symptom of hypertension attributed to renal artery occlusion. In some cases reviewed by Deming, the ischemic kidney apparently was putting out little or no urine. Hence, Deming suggested that polyuria resulted from a direct action on the normal kidney of renin released by the ischemic kidney. Some experimental evidence for such an effect of renin is available (63, 64).

However, Dollery and associates (65) and Laidlaw *et al.* (66) have recently described several cases in which polyuria and hypertension due to unilateral renal artery occlusion were associated with potassium depletion as well as other features suggesting hyperaldosteronism. The polyuria seen in these cases can be attributed to the potassium depletion. A similar explanation may apply to the patients referred to by Deming (61) and Margolin *et al.* (62). Sufficient data are not given to evaluate the possibility of potassium depletion in these cases.

Sickle-Cell Anemia

A specific defect in urine concentrating ability occurs in patients with sickle-cell anemia or trait. In infants and young children with sickle-cell anemia, maximum urine osmolality is markedly decreased, but Tc_{H_2O} is said to be relatively normal (67, 68). In older children and in adults, both maximum osmolality and Tc_{H_2O} are reduced (67). Maximum osmolality is subnormal even in individuals with sickle-cell trait who are not anemic (68, 69, 70). This is said to be a more uniform finding in adults than in children (68).

It was at first suggested that the impairment of urine-concentrating ability is the result of a genetically determined defect in the kidney. However, Keitel, Thompson & Itano (69) showed that the concentrating defect can be reversed by multiple transfusions of normal blood sufficient to clear nearly all sickle cells from the circulation. In four young children, maximum osmolality became normal after one month of transfusions; in an older child, a somewhat longer period was required. Glomerular filtration rate, filtration fraction, Tm_{PAH}, and solute excretion did not change in two subjects in whom these functions were studied. The concentrating defect was not reversed by multiple transfusions in two young adults. The defect is presumably not the result of anemia alone; it occurs in the absence of anemia in patients with sickle-cell trait (68, 69, 70) and is not present in patients with a number of other kinds of severe anemias (67, 69).

not given. Kleeman *et al.* (58) found a greater impairment of urine-concentrating ability in 13 patients with pyelonephritis than would be anticipated from the calculated degree of relative solute diuresis. The general trend of the relationship between maximum urine osmolality and serum creatinine was similar in groups of patients with pyelonephritis and glomerulonephritis. However, when the serum creatinine was less than 2 mg. per 100 ml., a definite concentrating defect was sometimes seen in patients with pyelonephritis, but never in those with glomerulonephritis. Moreover, four patients with pyelonephritis excreted urine persistently hypotonic to plasma in spite of a maximal antidiuretic stimulus, a phenomenon which did not occur in the patients with glomerulonephritis or nephrosclerosis.

Experimental evidence also suggests that pyelonephritis may cause relatively isolated damage to the concentrating mechanism. As already discussed, Bricker *et al.* (45) noted only slight decreases in maximum urine osmolality in experimental unilateral pyelonephritis in dogs. However, Shapiro and his co-workers (59) found striking decreases in maximum urine osmolality in rats with chronic enterococcal pyelonephritis when blood urea values were normal. Histologic studies showed marked damage to the renal medulla. A report in abstract form by Beck *et al.* (60) also describes a marked depression of maximum urine osmolality with normal blood urea levels in experimental chronic pyelonephritis in rats.

Such data as are available suggest that diluting ability is affected later and less severely than concentrating ability in pyelonephritis. In the study by Winberg (54) mentioned previously, infants and children with impaired urine concentrating ability during acute pyelonephritis were able to dilute urine normally. Brod (57) found that nearly all of his patients with chronic pyelonephritis could dilute urine to a specific gravity of 1.002 or less, including even those patients who were unable to achieve a maximum specific gravity over 1.012. Since specific gravity is a very rough measure of osmolality in the range of maximum urine dilution (40 to 100 mOsm/kg. H_2O), these observations are of debatable significance. The studies of Kleeman *et al.* (58) showed impaired ability to dilute urine in patients with pyelonephritis, as well as impaired concentrating ability. However, the diluting defect was thought to be entirely explicable by a relative osmotic diuresis, whereas the concentrating defect was not, as discussed previously.

The results summarized in this section thus suggest strongly, but do not definitely prove, that the concentrating mechanism may be specifically damaged in pyelonephritis, but the diluting mechanism may remain relatively intact. Since the final diluting operation is thought to occur in the distal convolutions, these observations suggest that this tubular segment is relatively intact. It would then follow that the medulla must be the site of the defect in concentration. This would be in accord with the evidence from pathology that pyelonephritis tends to damage medullary structure initially and most severely (58).

in obstructive hyposthenuria, no more informative statement as to the underlying mechanisms can be offered. In those cases caused by reversible obstruction the "water-losing" syndrome improved after the obstruction was corrected.

Electrolyte Disturbances

Disturbances of the concentrating mechanism occur in clinical and experimental states of potassium depletion (5 to 9), and of hypercalcemia (10 11), but these will not be discussed here.

RENAL ACIDOSIS

In a general discussion of the nature of renal acidosis (81) it was pointed out that the defect consists fundamentally of a failure of the renal tubules to excrete acid efficiently. Resort to such traditional concepts as "retention of acid anions" or loss of "base-sparing" capacity in reference to the mechanism of acidosis not only does violence to modern ideas of the nature of acids and bases (82, 83), but also tends to obscure the mechanisms involved in the tubular regulation of acid-base balance. No attempt will be made here to summarize the extensive body of experimental data concerning these mechanisms; authoritative reviews are available elsewhere (84 to 88). Suffice it to say that three tubular operations are involved: (*a*) The removal of almost all the filtered bicarbonate; (*b*) the addition of free ammonia or ammonium ion to the distal tubular fluid; and (*c*) the acidification of the urine buffers in the distal tubule, which results in the formation of "titratable acid." Present evidence strongly supports the hypothesis that all three processes involve the active reabsorption of a sodium ion in exchange for a hydrogen ion. The net excretion of acid by the kidney is measured by the sum of ammonium plus titratable acid, minus any bicarbonate remaining in the urine.

Types of acidosis.—It is customary to distinguish between two types of renal acidosis (89). By the term "uremic acidosis" is meant the acidosis which usually develops in the end stages of any type of generalized renal failure. Marked azotemia is the rule. The reduction in plasma bicarbonate is usually accompanied by significant rises in plasma phosphate and sulfate and, as most recently demonstrated by Seligson's studies (90), a variety of organic acid anions also accumulate. Chloride concentration is usually normal or, if there is hyponatremia, the concentration of chloride may be low. Serum potassium tends to be elevated (91). Although blood pH may be very low, respiratory compensation is often relatively poor as compared to that found in diabetic acidosis of equivalent severity (92). Obvious hyperventilation is not often observed in chronic uremic acidosis even when severe. This may reflect either a "toxic" effect of uremia on the respiratory center or simply an adaptation to the chronicity of the condition. So-called "tubular acidosis" usually refers to a relatively rare form of renal acidosis which occurs in a number of unifactorial or multifactorial renal tubular diseases in infants, children, or adults (1 to 5). Characteristically, there is little or no azotemia.

No complete explanation is available for the association of a urine-concentrating defect with circulating sickle cells. However, the experiments of Rennie, Reeves & Pappenheimer (71) in dogs suggest that oxygen tension may be much lower in the medulla of the kidney than in the cortex or in other organs. This would be conducive to sickling and subsequent circulatory obstruction in the vascular loops of the medulla. Functional and initially reversible changes in medullary circulation might in time lead to permanent alterations in medullary structure. Mostofi *et al.* (72) have described the striking pathological feature in the kidneys of 22 young adults with sickle-cell anemia as "severe stasis in peritubular capillaries . . . most marked in the medulla." Extravasation of blood and destruction of tubular epithelium apparently were later developments.

Acquired Nephrogenic Diabetes Insipidus

The persistent excretion of urine hypotonic to plasma in spite of the administration of maximal amounts of vasopressin has been named "nephrogenic diabetes insipidus." The congenital form of this syndrome will not be considered in this review, but it has been recently discussed (4, 13, 73).

As previously noted, nephrogenic diabetes insipidus occasionally occurs in the course of pyelonephritis (58). In the cases reported by Kleeman *et al.* (58, 74), GFR was markedly reduced so that an extreme relative solute diuresis was probably present. Under these conditions hypotonic urine may escape the distal convoluted tubules even when antidiuretic hormone activity is maximal (75, 76), and this phenomenon may play a role in the nephrogenic diabetes insipidus of pyelonephritis. If, as stated by Kleeman *et al.* (58), nephrogenic diabetes insipidus does not occur in the course of glomerulonephritis or nephrosclerosis, specific tubular damage in pyelonephritis may also play a part. Roussak & Oleesky (77) have described two cases of vasopressin-resistant diabetes insipidus under the title of "Water-Losing Nephritis." One case was associated with multiple myeloma and the second had prostatic hypertrophy and hydronephrosis. The patient with myeloma was hypercalcemic and this could well have explained the hyposthenuria (11). Also, the GFR was severely reduced and a marked relative solute diuresis per nephron may well have been present.

The occurrence of nephrogenic diabetes insipidus in association with urinary tract obstruction, as in Roussak & Oleesky's second case, has since been reported several times. Earley (78) described an infant with lower urinary tract obstruction in whom striking hyposthenuria and polyuria unresponsive to vasopressin were present. Winberg (79) reported that two children with lower urinary tract obstruction had vasopressin-resistant hyposthenuria. In their study of salt-wasting which follows the relief of obstructive uropathy, Bricker *et al.* (80) made no direct tests of concentrating or diluting function. Of interest, however, was the fact that one patient excreted urine hypotonic to plasma in spite of the administration of small amounts of vasopressin. Although tubular damage undoubtedly is involved

Although simple reduction in tubular mass or blood supply may be the ultimate explanation for the failure of compensatory excretion of ammonium in renal acidosis, other factors which might influence the production or transfer of ammonium need consideration. Unfortunately, pertinent data are scarce. The cellular factors essential for the synthesis of ammonia in man have not been clearly identified nor has there yet appeared any adequate biochemical explanation for the variations in ammonium excretion which normally can occur under physiological stimuli even without changes in urine pH. There is direct evidence in rats (101, 102) and in guinea pigs (103) that chronic acidosis induces increased glutaminase activity in the kidney; such is apparently not the case in dogs (104), and there is only inferential evidence suggesting adaptation of ammonia-producing enzymes in man (105). No analyses of renal tissue for these enzymes in uremic patients have yet been reported. Without much more information about the intermediary metabolism and enzymatic activity of renal tubular cells in health and disease, an adequate assessment of the role of possible tubular metabolic defects in the pathogenesis of uremic acidosis is impossible.

Finally, it should be noted that ammonium excretion may, to some degree, be dependent upon the factors which determine distal sodium transport (106). Uremic patients usually have at least some impairment of sodium conservation which could in part reflect defective distal transport mechanisms. The increased osmotic load per nephron which probably characterizes the uremic kidney might also be important, but available evidence (107) does not suggest any inhibitory effect of osmotic diuresis on ammonium excretion in normal man.

Titratable acid.—Although uremic patients are ultimately capable of acidifying their urine, they seem to require a greater degree of systemic acidosis to accomplish this than do normal subjects (97). To some extent, this tends to reduce the efficiency with which they can defend against acidosis, because the ultimate rise in titratable acidity will be delayed, as will also any increment in ammonium excretion. Wrong & Davies (98) have observed in patients with general renal failure (but no acidosis) that the excretion of phosphate on an unmeasured diet following a standard NH_4Cl load was significantly less than in normal controls. Other observations (108, 109) have also suggested but not conclusively demonstrated that urinary phosphate excretion tends to be low in advanced renal failure, possibly because relatively more phosphorus is excreted in the stool (108). If this is generally true for uremic acidotic patients, reduced urinary phosphate would help to explain why the excretion of titratable acid may, in some cases, be low despite very acid urines (97, 98). Most uremic patients, however, apparently can excrete normal amounts of titratable acid (93).

Bicarbonate-wasting.—In addition to the impairment in acid excretion manifested by patients with uremic acidosis another defect in acid-base regulation has been recently described. Schwartz *et al.* (97) have reported that in five of twelve patients there was a defect in bicarbonate reabsorption

and also no increase in plasma concentration of phosphate or sulfate. Hyperchloremia is the rule.

The terminology is perhaps unfortunate, since all renal acidosis is, in one sense, "tubular." Moreover, as will be discussed below, many of the clinical and physiological distinctions between the two types tend to become blurred upon closer examination. Nevertheless, it is still useful to consider these entities separately, in order to define and compare their characteristics and to summarize what is known about their mechanisms.

Uremic Acidosis

Ammonium excretion.—Studies by Palmer & Henderson (93), Gamble *et al.* (94), Van Slyke *et al.* (95), and Linder (96) established many years ago that ammonium excretion is deficient in azotemic patients, and that the capacity to increase ammonium excretion in response to acidifying salts is severely limited. Palmer & Henderson (93) showed, furthermore, that the ability to acidify the urine is preserved. More recent studies (97, 98) have confirmed this failure to excrete ammonium despite normal urine acidity. If the pH gradient between tubular cells and the urine is conceived as the major determinant of the transfer of cellular ammonia into the urine (99) then these observations could be taken to mean that the defect probably lies in the total rate of production of ammonia within tubular cells, rather than in its transport.

Little is known about the factors which limit ammonia production in renal disease. In patients with various types of renal disease, Wrong & Davies (98) found a rough proportionality between the excretion of ammonium after NH_4Cl and the glomerular filtration rate. They suggested that this might reflect a proportionate reduction in the blood supply to the tubules or in the number of functioning nephrons. Morrin, Bricker & Kime (100) have also found that in experimental unilateral or bilateral renal disease in dogs, a normal relationship between ammonium excretion and GFR is preserved. However, Kleeman *et al.* (58) refer to their unpublished evidence that in patients with pyelonephritis ammonium excretion factored by GFR is, in fact, significantly reduced, and it remains to be determined whether this is a functional characteristic of pyelonephritis in man. Whatever the case, interpretation of such data is very difficult. The precise relationship of GFR to the functioning mass or the blood supply of the ammonia-secreting portion of the nephron is unknown. Furthermore, quantitative comparisons of ammonium excretion need to be related to some standard stimulus. This condition is not satisfied by simply comparing individuals with similar degrees of extracellular acidosis, or by giving a standard load of NH_4Cl. There might still be differences in intracellular conditions or in the absorption and excretion of chloride which would affect the ammonium response. Chloride excretion in response to NH_4Cl also is said to be proportional to GFR (98); if so, it may be that the excreted anion load limits the ammonium excretion in these studies, rather than the reverse.

those fixed anions usually kept at low plasma concentrations. As glomerular insufficiency and uremia progressed, one would expect to see the hyperchloremia disappear and "unmeasured anions" rise.

Precisely this sequence of events has been reported by Lathem (118) in several patients with chronic pyelonephritis who had none of the other biochemical stigmata of "tubular acidosis." This is apparently not an unusual phenomenon, for Kleeman, Hewitt & Guze (58) found an "absolute or relative increase in the concentration of chloride in the serum" in approximately one-third of their acidotic pyelonephritic patients. Our own experience has been quite similar: of 32 adult patients with acidosis and chronic pyelonephritis, none of whom had other biochemical signs of "tubular acidosis," we have observed hyperchloremic acidosis without significant retention of "unmeasured anions" in 12. At the time they were hyperchloremic only three of these patients had BUN levels greater than 100 mg. per 100 ml. We have also seen occasional examples of hyperchloremic acidosis in patients with subacute or chronic glomerulonephritis, intercapillary glomerulosclerosis, and polycystic renal disease. In all of these cases, hyperchloremia tended to disappear when azotemia became more severe and "unmeasured anions" rose.

The mechanisms responsible for the reciprocal relationship between plasma chloride on the one hand and plasma bicarbonate and "unmeasured anions" on the other, are not entirely clear. Some insight into the possible nature of the reciprocity between chloride and bicarbonate in non-uremic acidosis is gained from the fact that proximal reabsorption of chloride passively follows the electrical gradient established by active sodium transport (119). Reduction in the amount of hydrogen secretion with continued active sodium transport might therefore tend to increase the electrical driving force on chloride and thus increase the proportion of chloride to sodium in the reabsorbate (88). In uremic acidosis, this proportion might tend to fall if the reabsorption of "unmeasured anions" from the filtrate were to be increased relative to that of sodium. Clarification of this problem will require much more detailed information about anion transport in various stages of renal disease than is presently available.

Renal Tubular Acidosis

Clinical and biochemical features.—So-called renal tubular acidosis (RTA) in adults is a rare form of tubular disease which characteristically produces hyperchloremic acidosis and a distinctive clinical and biochemical syndrome. In almost all patients there are a number of associated tubular defects, most frequently hypercalcuria and renal potassium-wasting. The former leads often to nephrolithiasis; the latter often results in severe potassium depletion. Hypophosphatemia and osteomalacia are commonly present, and calcification of the renal medulla is also a frequent, though not invariable, feature. Less commonly there may be renal glucosuria or amino aciduria (4).

RTA is often associated with chronic pyelonephritis, particularly in

which caused significant urinary losses of bicarbonate, even while plasma bicarbonate levels were falling below normal. This "bicarbonate-wasting" ceased only after plasma concentratioos were very low; in some cases the urinary loss was adequate to account for half or more of the drop in extracellular bicarbonate. "Bicarbonate-wasting" would not be evident in such uremic patients unless they were receiving alkali, which probably explains why this functional disorder bas not received attention in the past. Measurements of bicarbonate reabsorption during acute loading with bicarbonate, such as carried out by Roberts and her associates (110) also would not readily reveal the defect because the reduction in reabsorptive rate required to produce the daily excretion of significant quantities of bicarbonate need be quite small.

No explanation is at hand for the bicarbooate-wasting defect. A reduction in tubular carbonic anhydrase activity could explain this phenomenon (97), but there is no positive evidence for this. It has been reported that the bicarbonate diuresis in response to a standard dose of acetazolamide is reduced in patients with uremic acidosis (111). In view of the direct relationship between plasma bicarbonate level and the effect of this enzyme inhibitor on bicarbonate reabsorption (112) such observations provide no critical information about the activity of carbonic anhydrase in diseased tubules. Here again, direct tissue analyses for enzyme activity would be of considerable interest.

Osmotic loading of remaining nephrons, which has often been invoked to explain sodium-wasting in chronic renal failure (113, 114), is not apt to be the explanation for bicarbonate-wasting as well During the latter phenomenon, the excretion of bicarbonate may far exceed that of chloride (97). With osmotic loading in normal kidneys, chloride excretion is increased relatively more than that of bicarbonate (115, 116, 117).

Hyperchloremic ocidosis in the common forms of renal foilure.—Hyperchloremia is not ordinarily associated with the acidosis resulting from the common causes of renal failure. Plasma bicarbonate is usually replaced by "unmeasured" anions such as phosphate, sulfate, and organic acids, thus appearing to lend plausibility to the archaic notion that the acidosis of uremia is somehow caused by the retention of these anions. Hyperchloremia, on the other hand, is usually considered to be the hallmark of tubular acidosis, and quite different mechanisms of pathogenesis are thougbt to be involved. However, it has already been noted (81) that failure of one or more tubular mechanisms for acid excretion must account for both types of renal acidosis and it has been further suggested that the level of chloride in plasma is probably inversely related to the extent to which reduction of glomerular filtration rate elevates the concentration of "unmeasured anions." It would therefore be predictable that hyperchloremic acidosis might occur as a phase in the development of various renal diseases, when the tubular acid-secreting function has been sufficiently impaired to cause acidosis but when glomerular function has not yet been reduced enough to cause significant retention of

of adult or infantile RTA, were nevertheless considerably higher than normal acidotic controls who had maximally acid urines both before and after the phosphate infusion. Furthermore, none of the patients excreted acid urines prior to the phosphate load, despite very low plasma bicarbonate levels. Acidifying responses to infused anion loads may be expected to vary with the tubular handling of sodium (106) and with the permeability of the anion (125). Hence, the modest reduction in urine pH during phosphate infusion cannot be construed as necessarily indicating normal distal tubular capacity to establish gradients of hydrogen ion, although these observations do show that the acidifying defect may be relatively slight in some patients.

Reynolds (122), Burnett & Williams (3), and Wrong & Davies (98) have suggested that the essential defect in RTA consists of an inability to acidify the urine without any underlying defect in ability to produce ammonia or to exchange sodium for hydrogen at less than maximal hydrogen ion gradients. Nothing is known about the exact nature of the acidifying defect. Deficiency of carbonic anhydrase has often been suggested as a possible explanation, based mainly on the superficial analogy between RTA and the effects of continuous administration of a carbonic anhydrase inhibitor such as acetazolamide. Blunted responses to this agent in patients with RTA (111, 122, 126, 127) add little strength to this hypothesis since, as pointed out before, response is normally blunted in the presence of metabolic acidosis. Against the carbonic anhydrase idea, however. is the fact that "bicarbonate-wasting" is not a prominent feature of this disease. Recently. Yaffe, Craig & Fellers (127) have reported that carbonic anhydrase activity was normal in renal tissue taken by biopsy from a child with RTA. They also found normal glutaminase activity and they suggested a defect in "energy production" within tubular cells as a possible cause of inability to acidify. Since potassium depletion may somewhat impair urine acidifying ability (128). the possibility exists that this may play a role in patients with RTA who are potassium-depleted. However, occasional patients do not become potassium-depleted, but nevertheless fail to acidify normally (98, 129, 130). Furthermore, the acidifying defect in potassium depletion is neither as invariable nor as severe as that manifested in RTA.

those patients with renal stones. In some cases, a slowly progressive generalized renal insufficiency develops, sometimes with hypertensive cardiovasuclar disease. In that event, the biochemical features of hyperchloremic tubular acidosis may well ultimately be replaced by those of uremia. Whether pyelonephritis is of importance in producing the tubular damage that results in RTA or is simply a frequent complication, cannot be definitely decided in many cases. This is particularly true of those in whom the disease appears to occur *de novo* in adult life. That at least a substantial number of cases are of hereditary origin is strongly suggested by the frequency of familial involvement (98) and by the frequency of onset in infancy or childhood.

Tubular acid-base regulation.—The distinctive functional defect in RTA of the adult is the inability to acidify the urine much below a pH of 6.0, even when there is no complicating urinary infection. Numerous studies (97, 98, 120, 121, 122) have demonstrated that the urine fails to become acidified even when the plasma bicarbonate concentration is reduced to very low levels either spontaneously or by the administration of ammonium chloride. Ammonium excretion is either normal or slightly high in relation to the pH of the urine (98, 122, 123), but patients with RTA show very little rise in ammonium output when challenged with acid loads (97, 98, 120, 122). In two patients studied by Schwartz *et al.* (97) a slight bicarbonate-wasting defect was demonstrated when the plasma bicarbonate level was raised to normal, but the defect was not as great as has been observed in occasional patients with uremic acidosis. Infusion of bicarbonate has been reported to result in relatively little immediate diuresis of bicarbonate; the calculated rates of reabsorption were within normal limits (121, 122). It should be re-emphasized here that acute observations of this sort may be misleading; relatively unimpressive changes in rates of reabsorption per minute may be enough to determine significant variations in total daily excretion of bicarbonate. Since the urine is always alkaline there is, in a sense, a constant small leakage of bicarbonate in the urine even when the plasma bicarbonate remains at a constant low level (97, 98, 121, 122). The excretion of titratable acid is reduced, mainly due to the constant alkalinity of the urine and not to any reduction in phosphate excretion. A number of investigators (98, 121, 122) have shown that infusion of neutral phosphate with resultant increase in phosphate excretion will not change urine pH significantly and will therefore increase the excretion of titratable acidity in direct proportion to the phosphaturia. There does not, therefore, seem to be any readily demonstrable limit to the distal exchange of sodium for hydrogen in the formation of titratable acid even though the capacity to establish hydrogen ion gradients is significantly impaired.

In contrast to the observations summarized above, Latner & Burnard (124) observed in six infants with RTA that rapid infusion of neutral phosphate resulted in acidification of the urine and significant increments in ammonium excretion. Urine pH during the infusion ranged from 5.09 to 5.80; these values, though lower than most of those reported in other cases

Kime, S. W., Jr., *Am. J. Med.*, 28, 77–98 (1960)
48. Baldwin, D. S., Berman, H. J., Heinemann, H. O., and Smith, H. W., *J. Clin. Invest.*, 34, 800–7 (1955)
49. Dorhout Mees, E. J., *Brit. Med. J.*, I, 1156–58 (1959)
50. Dorhout Mees, E. J., *Brit. Med. J.*, I, 1159–60 (1959)
51. Franklin, S. S., Niall, J. F., and Merrill, J. P., *J. Clin. Invest.*, 38, 1005 (1959) (Abstr.)
52. Levitt, M. F., Levy, M. S., and Polimeros, D., *J. Clin. Invest.*, 38, 463–73 (1959)
53. Kleeman, C. R., Adams, D. A., and Maxwell, M. H., *Clin. Research*, 7, 77–78 (1959) (Abstr.)
54. Winberg, J., *Acta Pediat.*, 48, 577–89 (1959)
55. Kaitz, A. L., *Clin. Research*, 8, 229 (1960) (Abstr.)
56. Raaschou, F., *Studies of Chronic Pyelonephritis* (Ejnar Munksgaard, Copenhagen, 260 pp., 1948)
57. Brod, J., *Lancet*, I, 973–81 (1956)
58. Kleeman, C. R., Hewitt, W. L., and Guze, L. B., *Medicine*, 39, 3–116 (1960)
59. Shapiro, A. P., Braude, A. I., and Siemienski, J., *J. Clin. Invest.*, 38, 1228–40 (1959)
60. Beck, D., Freedman, L. R., Levitin, H., Ferris, T. F., and Epstein, F. H., *Clin. Research*, 8, 226 (1960) (Abstr.)
61. Deming, Q. B., *Arch. Internal Med.*, 93, 197–204 (1954)
62. Margolin, E. G., Merrill, J. P., and Harrison, J. H., *New Engl. J. Med.*, 256, 581–88 (1957)
63. Goldblatt, H., in *The Renal Origin of Hypertension* (Cannon, P. R., Ed., Charles C Thomas, Springfield, Ill., 126 pp., 1948)
64. Hughes-Jones, N. C., Pickering, G. W., Sanderson, P. H., Scarborough, H., and Vanderbroucke, J., *J. Physiol.*, 109, 288–307 (1949)
65. Dollery, C. T., Shackman, R., and Shillingford, J., *Brit. Med. J.*, II, 1367–71 (1959)
66. Laidlaw, J. C., Yendt, E. R., and Gornall, A. G., *Metabolism*, 9, 612–23 (1960)
67. Heinemann, H. O., and Cheung, M. W., *J. Lab. Clin. Med.*, 49, 923–27 (1957)
68. Whitten, C. F., and Younes, A. A., *J. Diseases Children*, 96, 446–49 (1958) (Abstr.)
69. Keitel, H. G., Thompson, D., and Itano, H. A., *J. Clin. Invest.*, 35, 998–1007 (1956)
70. Zarafonetis, C. J. D., McMaster, J. D., Molthan, L., and Steiger, W. A., *Am. J. Med. Sci.*, 232, 76–82 (1956)
71. Rennie, D. W., Reeves, R. B., and Pappenheimer, J. R., *Am. J. Physiol.*, 195, 120–32 (1958)
72. Mostofi, F. K., Vorder Bruegge, C. F., and Diggs, L. W., *Arch. Pathol.*, 63, 336–51 (1957)
73. Robinson, M. G., and Kaplan, S. A., *J. Diseases Children*, 99, 164–74 (1960)
74. Kleeman, C. R., and Epstein, F. H., *Am. J. Med.*, 23, 488–92 (1957)
75. Orloff, J., Wagner, H. N., Jr., and Davidson, D. G., *J. Clin. Invest.*, 37, 458–64 (1958)
76. Raisz, L. G., Au, W. Y. W., and Scheer, R. L., *J. Clin. Invest.*, 38, 1725–32 (1959)
77. Roussak, N. J., and Oleesky, S., *Quart. J. Med.*, 23, 147–64 (1954)
78. Earley, L. E., *New Engl. J. Med.*, 255, 600–5 (1956)
79. Winberg, J., *Acta Pediat.*, 48, 149–63 (1960)
80. Bricker, N. S., Shwayri, E. I., Reardan, J. B., Kellogg, D., Merrill, J. P., and Holmes, J. H., *Am. J. Med.*, 23, 554–64 (1957)
81. Schwartz, W. B., and Relman, A. S., *New Engl. J. Med.*, 256, 1184–86 (1957)
82. Relman, A. S., *Am. J. Med.*, 17, 435–37 (1954)
83. Christensen, H. N., *Diagnostic Biochemistry*, (Oxford Univ. Press New York, N. Y., 291 pp., 1959)
84. Gilman, A., and Brazeau, P., *Am. J. Med.*, 15, 765–70 (1953)
85. Pitts, R. F., *Harvey Lectures*, 48, 172–209 (1952–53)
86. Orloff, J., *Yale J. Biol. and Med.*, 29, 211–28 (1956)
87. Smith, H. W., *Principles of Renal Physiology* (Oxford Univ. Press, New York, N. Y., 237 pp., 1956)
88. Pitts, R. F., *Am. J. Med.*, 24, 745–63 (1958)
89. Elkinton, J. R., *Am. J. Med.*, 28, 165–68 (1960)
90. Seligson, D., Bluemle, L. W., Jr., Webster, G. D., Jr., and Senesky, D., *J. Clin. Invest.*, 38, 1042–43 (1959) (Abstr.)
91. Schwartz, W. B., *New Engl. J. Med.*, 253, 601–8 (1955)
92. Møller, B., *Acta Med. Scand.*, *Suppl. No. 348*, 11–340 (1959)

LITERATURE CITED

1. Payne, W. W., *Pediatrics*, 17, 84–92 (1956)
2. Piel, C. F., *Pediatrics*, 20, 337–57 (1957)
3. Burnett, C. H., and Williams, T. F., *Arch. Internal Med.*, 102, 881–90 (1958)
4. Mudge, G. H., *Am. J. Med.*, 24, 785–804 (1958)
5. Stanbury, S. W., *Advances in Internal Med.*, 9, 231–82 (1958)
6. Conn, J. W., and Johnson, R. D., *Am. J. Clin. Nutrition*, 4, 523–28 (1956)
7. Milne, M. D., Muehrcke, R. C., and Heard, B. E., *Brit. Med. Bull.*, 13, 15–18 (1957)
8. Relman, A. S., and Schwartz, W. B., *Am. J. Med.*, 24, 764–73 (1958)
9. Welt, L. G., Hollander, W., Jr., and Blythe, W. B., *J. Chronic Diseases*, 11, 213–54 (1960)
10. Kushner, D. S., *Am. J. Clin. Nutrition*, 4, 561–79 (1956)
11. Epstein, F. H., *J. Chronic Diseases*, 11, 255–77 (1960)
12. de Wardener, H., *J. Chronic Diseases*, 11, 199–212 (1960)
13. Holliday, M. A., *J. Pediat.*, 57, 23–35 (1960)
14. Wirz, H., Hargitay, B., and Kuhn, W., *Helv. Physiol. et Pharmacol. Acta*, 9, 196–207 (1951)
15. Wirz, H., *Proc. Symposium Colston Research Soc., 8th Symposium*, 157–66 (1957)
16. Wirz, H., *Helv. Physiol. et Pharmacol. Acta*, 14, 353–62 (1956)
17. Gottschalk, C. W., and Mylle, M., *Am. J. Physiol.*, 196, 927–36 (1959)
18. Ullrich, K. J., *Circulation*, 21, 869–74 (1960)
19. Ullrich, K. J., Drenckhahn, F. O., and Jarausch, K. H., *Arch. ges. Physiol.*, 261, 62–77 (1955)
20. Levinsky, N. G., Davidson, D. G., and Berliner, R. W., *J. Clin. Invest.*, 38, 730–40 (1959)
21. Berliner, R. W., Levinsky, N. G., Davidson, D. G., and Eden, M., *Am. J. Med.*, 24, 730–44 (1958)
22. Lamdin, E., *Arch. Internal Med.*, 103, 644–71 (1959)
23. Gottschalk, C. W., *Circulation*, 21, 861–68 (1960)
24. Gamble, J. L., McKhann, C. F., Butler, A. M., and Tuthill, E., *Am. J. Physiol.*, 109, 139–54 (1934)
25. Epstein, F. H., Kleeman, C. R., Pursel, S., and Hendrikx, A., *J. Clin. Invest.*, 36, 635–41 (1957)
26. Meroney, W. H., Rubini, M. E., and Blythe, W. B., *Ann. Internal Med.*, 48, 562–73 (1958)
27. Levinsky, N. G., and Berliner, R. W., *J. Clin. Invest.*, 38, 741–48 (1959)
28. Epstein, F. H., Kleeman, C. R., and Hendrikx, A., *J. Clin. Invest.*, 36, 629–34 (1957)
29. de Wardener, H. E., and Herxheimer, A. W., *J. Physiol.*, 139, 42–52 (1957)
30. Barlow, E. D., and de Wardener, H., *Quart. J. Med.*, 28, 235–58 (1959)
31. Edelmann, C. M., Jr., and Barnett, H. L., *J. Pediat.*, 56, 154–79 (1960)
32. Edelmann, C. M., Jr., Barnett, H. L., and Troupkou, V., *J. Clin. Invest.*, 39, 1062–69 (1960)
33. Miles, B. E., Paton, A., and de Wardener, H. E., *Brit. Med. J.*, II, 901–5 (1954)
34. Levinsky, N. G., Davidson, D. G., and Berliner, R. W., *Am. J. Physiol.*, 196, 451–56 (1959)
35. Jaenike, J. R., and Waterhouse, C., *Clin. Research*, 7, 272 (1959)
36. Hayman, J. M., Jr., Shumway, N. P., Dumke, P., and Miller, M., *J. Clin. Invest.*, 18, 195–212 (1939)
37. Mudge, G. H., Foulks, J., and Gilman, A., *Am. J. Physiol.*, 158, 218–30 (1949)
38. Rapoport, S., Brodsky, W. A., West, C. D., and Mackler, B., *Am. J. Physiol.*, 156, 433–42 (1949)
39. Wesson, L. G., Jr., and Anslow, W. P., Jr., *Am. J. Physiol.*, 170, 255–69 (1952)
40. Smith, H. W., *The Kidney. Structure and Function in Health and Disease* (Oxford Univ. Press, New York, N. Y., 1049 pp., 1951)
41. Platt, R., *Brit. Med. J.*, I, 1313–17 (1952)
42. Strauss, M. B., *Body Water in Man* (Little, Brown & Co., Boston, Mass., 286 pp., 1957)
43. Welt, L. G., *Yale J. Biol. and Med.*, 29, 299–315 (1956)
44. de Wardener, H. E., *J. Chronic Diseases*, 11, 199–212 (1960)
45. Bricker, N. S., Dewey, R. R., Lubowitz, H., Stokes, J., and Kirkensgaard, T., *J. Clin. Invest.*, 38, 516–23 (1959)
46. Bricker, N. S., Kime, S. W., Jr., Morrin, P. A. F., and Orlowsky, T., *J. Clin. Invest.*, 39, 864–75 (1960)
47. Bricker, N. S., Morrin, P. A. F., and

UNTOWARD REACTIONS TO ANTIMICROBIAL AGENTS[1]

By H. J. Simon, M.D., Ph.D., and L. A. Rantz, M.D.
Stanford University School of Medicine, Stanford Medical Center, Palo Alto, California

Untoward reactions to antimicrobial agents are of three types. One, often related to the dose, is caused by the direct toxic action of the drug. Another, less clearly correlated with the amount administered, is usually regarded as due to allergic hypersensivity. A third type of reaction is caused by alterations in the normal flora of the body with subsequent superinfection by organisms resistant to the antimicrobial agent that is being administered.

It is the purpose of this review to analyze the nature, frequency of occurrence, and mechanisms of reactions to antimicrobial agents. Information which appeared between 1956 and June of 1960 has been included. Lack of space prevented the consideration of bacitracin, erythromycin, isonicotinic acid hydrazide, oleandomycin, penicillin, *para*-amino salicylic acid, polymyxin, and the sulfonamides. All have been in clinical use for many years and the untoward results of their administration are well defined.

Many other reviews have considered the literature on untoward reactions to the various antibacterial drugs (1 to 10). Problems arising from the unjustified or prophylactic use of antimicrobial agents, the emergence of drug-resistant microorganisms, and the danger to the population arising from the antimicrobial contamination of foodstuffs, are beyond the scope of this article.

The agents selected for discussion are presented alphabetically under their generic names. Those included are amphotericin-B, chloramphenicol, furaltadone, griseofulvin, kanamycin, neomycin, nitrofurantoin, novobiocin, ristocetin, the streptomycins, the tetracyclines, and vancomycin.

The more important untoward reactions are summarized in the table which appears at the end of this review. This table lists reactions to the antimicrobial agents which are discussed in the paper as well as the more important reactions to those agents which could not be discussed because of space limitation. Pertinent reference numbers for the latter are included in the table and the references themselves appear under their appropriate headings in the bibliographic appendix.

Amphotericin-B

Amphotericin-B is an agent with a high degree of activity against certain fungi associated with localized and systemic mycotic infections. It is only slightly soluble and is poorly absorbed from the gastrointestinal tract or from depots following intramuscular injection. For systemic therapy, there-

[1] The survey of the literature pertaining to this review was concluded in September, 1960.

93. Palmer, W. W., and Henderson, L. J., *Arch. Internal Med.*, 16, 109–31 (1915)
94. Gamble, J. L., Blackfan, K. D., and Hamilton, B., *J. Clin. Invest.*, 1, 359–88 (1925)
95. Van Slyke, D. D., Linder, G. C., Hiller, A., Leiter, L., and McIntosh, J. F., *J. Clin. Invest.*, 2, 255–88 (1926)
96. Linder, G. C., *Quart. J. Med.*, 20, 285–302 (1927)
97. Schwartz, W. B., Hall, P. W., 3rd, Hays, R. M., and Relman, A. S., *J. Clin. Invest.*, 38, 39–52 (1959)
98. Wrong, O., and Davies, H. E. F., *Quart. J. Med.*, 28, 259–313 (1959)
99. Orloff, J., and Berliner, R. W., *J. Clin. Invest.*, 35, 223–35 (1956)
100. Morrin, P. A. F., Bricker, N. S., and Kime, S. W., Jr., *J. Clin. Invest.*, 39, 1013–14 (1960) (Abstr.)
101. Davies, B. M. A., and Yudkin, J., *Biochem. J.*, 52, 407–12 (1952)
102. Rector, F. C., Jr., Seldin, D. W., and Copenhaver, J. H., *J. Clin. Invest.*, 34, 20–26 (1955)
103. Goldstein, L., Richterich, R., and Dearborn, E. H., *Proc. Soc. Exptl. Biol. Med.*, 93, 284–87 (1956)
104. Rector, F. C., Jr., and Orloff, J., *J. Clin. Invest.*, 38, 366–72 (1959)
105. Madison, L. L., and Seldin, D. W., *J. Clin. Invest.*, 37, 1615–27 (1958)
106. Schwartz, W. B., Jenson, R. L., and Relman, A. S., *J. Clin. Invest.*, 34, 673–80 (1955)
107. Beck, R. N., *Clin. Sci.*, 17, 37–42 (1958)
108. Liu, S. H., and Chu, H. I., *Medicine*, 22, 103–61 (1943)
109. Hodgkinson, A., and Pyrah, L. N., *Brit. J. Surg.*, 46, 10–18 (1958–59)
110. Roberts, K. E., Randall, H. T., Vanamee, P., and Poppell, J. W., *Metabolism*, 5, 404–18 (1956)
111. Kaye, M., *J. Clin. Invest.*, 34, 277–84 (1955)
112. Schwartz, W. B., Falbriard, A., and Relman, A. S., *J. Clin. Invest.*, 37, 744–51 (1958)
113. Bull, G. M., *Lancet*, I, 731–36, 777–81 (1955)
114. Stanbury, S. W., and Mahler, R. F., *Quart. J. Med.*, 28, 425–47 (1959)
115. Wesson, L. G., Jr., Anslow, W. P., Jr., and Smith, H. W., *Bull. N. Y. Acad. Med.*, 24, 586–606 (1948)
116. Goodyer, A. V. N., Welt, L. G., Darragh, J. H., Abele, W. A., and Meroney, W. H., *Proc. Soc. Exptl. Biol. Med.*, 86, 19–22 (1954)
117. Anslow, W. P., Jr., and Wesson, L. G., Jr., *Am. J. Physiol.*, 180, 605–11 (1955)
118. Lathem, W., *New Engl. J. Med.*, 258, 1031–36 (1958)
119. Giebisch, G., *Circulation*, 21, 879–91 (1960)
120. Albright, F., and Reifenstein, E. C., Jr., *The Parathyroid Glands and Metabolic Bone Disease*, 227–57 (Williams & Wilkins Co., Baltimore, Md., 393 pp., 1948)
121. Smith, L. H., Jr., and Schreiner, G. E., *J. Lab. Clin. Med.*, 43, 347–58 (1954)
122. Reynolds, T. B., *Am. J. Med.*, 25, 503–15 (1958)
123. Milne, M. D., Stanbury, S. W., and Thomson, A. E., *Quart. J. Med.*, 21, 61–82 (1952)
124. Latner, A. L., and Burnard, E. D., *Quart. J. Med.*, 19, 285–301 (1950)
125. Bank, N., and Schwartz, W. B., *J. Clin. Invest.*, 39, 970 (1960) (Abstr.)
126. Webster, G. D., Jr., and Huth, E. J., *J. Clin. Invest.*, 38, 1053 (1959) (Abstr.)
127. Yaffe, S. J., Craig, J. M., and Fellers, F. X., *Am. J. Med.*, 29, 168–75 (1960)
128. Clarke, E., Evans, B. M., MacIntyre, I., and Milne, M. D., *Clin. Sci.*, 14, 421–40 (1955)
129. Wilansky, D. L., and Schneiderman, C., *New Engl. J. Med.*, 257, 399–403 (1957)
130. Frick, P. G., Rubini, M. E., and Meroney, W. H., *Am. J. Med.*, 25, 590–99 (1958)

drug is diluted and carefully administered. Dosages of the order of 0.5 to 0.7 mg. every 48 to 72 hr. have, nevertheless, resulted in arachnoiditis on at least one occasion, but only after several months of treatment. Pain in the legs and back occasionally follows this form of therapy. This reaction seems to be controllable with elevation of the foot of the bed by ten inches for one hour after administration of the drug. The site of the lumbar puncture should be varied for each injection (3).

Intrapleural injection of amphotericin may give rise to most of the systemic effects of intravenous injection unless small doses are used. This method seems particularly indicated following pulmonary surgery for mycotic lesions caused by susceptible microorganisms (8).

Amphotericin has been injected directly into localized regions such as joints, subcutaneous lesions, bone, and lymph-node abscesses. The dose varies with the location of the lesion (1, 5, 6, 9). Systemic effects are slight, and local reactions are generally mild if the concentration is low.

CHLORAMPHENICOL

Chloramphenicol is an effective antimicrobial agent which, at first, seemed to be quite safe. Within two years however, blood dyscrasias were observed and, more recently, unusual reactions in infants have been reported.

Blood dyscrasias.—Several comprehensive reports have dealt extensively with the problem of chloramphenicol-induced blood dyscrasias (1 to 4). These reactions most commonly take the form of pancytopenia, although agranulocytosis and thrombocytopenia have also been documented on numerous occasions. A large number of these accidents occurred in individuals who had ready access to this drug and used it without adequate indication. Other patients were given frequent, short courses of chloramphenicol. Not every case of so-called chloramphenicol-induced bone marrow depression was clearly attributable to this agent. Many patients had been simultaneously exposed to other drugs which were, themselves, occasionally associated with these accidents. On the other hand, it must be appreciated that not all of the chloramphenicol-induced blood dyscrasias have been reported.

Between 1953 and 1960 the Registry on Blood Dyscrasias was informed of 34 instances of pancytopenia in which chloramphenicol was the only drug administered (3). Another study of the same subject cites 31 cases which occurred between 1955 and 1957 (2). Twenty-three of these 31 cases terminated fatally. Only eight of the 31 cases had previously been reported to the Registry. One reaction per 10,000 patients treated may be a reasonable reflection of the actual incidence.

Chloramphenicol therapy in infants.—Premature infants were given chloramphenicol prophylactically alone or in combination with other antimicrobial agents after spontaneous rupture of the membranes (5). Other groups of premature infants received either no antimicrobial agents, or a

fore, this agent must be employed intravenously, intrathecally, or intrapleurally. It can be used topically for local therapy with reasonably good results.

Toxicity.—Most patients receiving amphotericin-B intravenously will experience reactions of varying severity consisting of fever, chills, beadacbe, nausea, vomiting, anorexia, and malaise (1, 2, 3). Such reactions usually subside with continued tberapy. It is advisable to begin with small doses, gradually increasing the amount until tbe desired schedule is achieved (1, 4 to 6). A number of investigators suggest that many nf the side reactions can be minimized if therapy is given nnly every nther day (3 to 6). This is rational because of the prolonged persistence of significant amphotericin concentrations in serum following a single dose. Cbills and fever can be minimized with premedication consisting of antipyretics and antihistaminic agents (1, 3, 6). Sometimes all of these measures fail, and therapy must be discontinued.

Azotemia bas been a frequent complication of amphotericin tberapy. Usually, there is no other evidence of a renal lesion, and the nitrogen retention regresses when the drug is discontinued (1, 3 to 5). Reducing the dose, or reverting to an alternate-day scheme of therapy may be enough to restore the renal function to normal or near-normal. Suitable serial tests of kidney function are mandatory in the management of patients receiving amphotericin systemically.

Chemical thrombophlebitis following the intravenous administration of amphotericin is quite common unless certain precautions are observed. Tbe concentration of the drug sbould not exceed 0.1 mg. per ml. The rate of the infusion should be slow. A small gauge needle should be employed, and the site of infusion should be rotated daily (3). It may be necessary to use an indwelling catheter situated in the vena cava if the above measures fail. Heparin in 10 or 20 mg. amounts added to the infusion may also be of benefit.

Individual case reports have commented on other and apparently uncommon side effects of amphotericin therapy. These have included anemia, chest pain, abdominal discomfort, gastrointestinal bleeding, flushing, anxiety, hypokalemia, apparent bepatic toxicity, and convulsions in infants. Single instances of the follnwing reactions have also been reported: drowsiness, generalized pain, blurring nf vision, urinary casts, transient leucopenia, a temporary increase in heart size, and generalized pruritus (1 to 7). In one instance, the rapid infusion of tbe drug (28 mg. in 40 min.) resulted in generalized convulsions, ventricular fibrillation, and cardiac arrest with recovery following a sharp blow on the chest (4).

To date, all toxic reactinns to intravenous amphotericin have been reversible upon discontinuatinn nf the drug. Reduction of dosage or intermittent cessation of therapy may be required on occasion, but permanent witbdrawal of therapy rarely seems necessary.

Intrathecal injections nf amphntericin are usually well tolerated if the

TABLE I *Continued*

Agent	Mucus Mem and Skin	G.I.	Blood Marrow	C.N.S.	Liver	Renal	Local	Fever	Anaphylactoid	Other	Comment	Frequency	Reference
Griseofulvin		×		×							Nausea Headache	F	(1 to 6) (7, 9, 12)
			×								Leucopenia	R	(1, 7, 9, 10)
										Potentiates alcohol		R	(9)
	+										Mild	R	All reports
	ƒ										Severe	R	(9, 11)
Neomycin Kanamycin						×					Liver— Renal— Colitic Bowel Perforation	F	(1 to 4)
				×							Ototoxic	F	(1, 5, 6, 9, 10)
										Intraperitoneal	Resp. Arrest	R	(11, 12)
	+											F	(14 to 17)
		×										F	(1, 19)
										Malabsorption		R	(14)
Nitrofurantoin	+							+				R	(3, 4)
		×										F	(3, 4)
			×							"Primaquine"		R	(1, 2)
Novobiocin	+	×										F	(1 to 6) (7, 8, 9)
			+									R	(7, 13 to 15, 16)
				ƒ								R	(17)
						?				Yellow	Yellow Serum	R	(10, 11, 12)
Penicillin	+	+						+				F	All reports
									+			R	(1 to 5, 8)
										L-E Phenomenon		R	(6, 7)
				×						Intrathecal Use		R	
Polymixin-B				×								F	(2, 3)
						×						F	(4)

TABLE I

UNTOWARD REACTIONS TO ANTIMICROBIAL AGENTS

Agent	Mucus Mem. and Skin	G.I.	Blood Marrow	C.N.S.	Liver	Renal	Local	Fever	Anaphylactoid	Other	Comment	Frequency	Reference**
Amphotericin-B								×			Early	F	(1 to 6)
													(7, 8, 9)
						×						F	(1, 3, 4, 5)
							×				Phlebitis	F	(3)
				×								F	(1 to 7)
			ƒ	ƒ	ƒ	ƒ				Cardiac Arrest		R	(1 to 7)
Bacitracin						×						F	(1)
Chloramphenicol	+	+										R	(2, 4)
			×									R	(1, 3, 4)
									+			R	(2, 4)
										Grey Disease	Infants	F	(5, 6)
Erythromycin Oleandomycin	+	×									N V, D	R F	(1 to 6) (1 to 6)
Spiramycin									ƒ			R	(7)
		×								Staph. enteritis		R	(3, 8, 9)
Furaltadone			+								Eosinophilia	F	(1)
			+							Leucopenia		R	(1)
		×									N, V	F	(1, 2)
										Antabuse Effect	Alcohol Intake	F	(2)
										Hemolysis		?	(1)
	+							+				R	(1)
				×							Ocular, Auditory, Periph.	F	(2, 3)
						ƒ					Azotemia	R	(1)

Key to Table:

× = Toxic Reaction F = Frequent
\+ = Hypersensitivity Reaction N = Nausea
ƒ = Isolated Case Reports V = Vomiting
R = Rare D = Diarrhea
** Numbers refer to appended bibliography by alphabetized sections.

combination of penicillin and streptomycin. Chloramphenicol was used in a dosage of 165 mg. per kg. per day. None of the prophylactic regimens protected the infants in any way, and all such treatment was associated with a higher morbidity than was found in the control group. Both study groups receiving chloramphenicol demonstrated a significantly greater mortality rate than either the control or the penicillin-streptomycin treated group.

The clinical courses of the chloramphenicol-treated infants who died were strikingly similar. The signs appeared in a characteristic sequence, and gave rise to the term "gray syndrome." Treatment was started within 12 hr. of birth. Each infant's condition was satisfactory for the next 48 hr. Then followed a sequence of vomiting, refusal to take food, respiratory distress, abdominal distension, cyanosis, and the passage of loose, green stools over the next four or five days. The infants became flaccid, their color was ashen, and they died in circulatory collapse. When therapy was discontinued before the infants became seriously ill, recovery usually ensued during the subsequent 24 to 36 hr.

This syndrome has occurred in both premature and full-term infants (5, 6). The rapidity of onset and severity of the disorder varied directly with the dosage of chloramphenicol and seemed most violent when therapy was started very early in life, especially in the premature group.

Chloramphenicol serum concentrations were found to be extremely high, apparently because of the prolonged persistence of the drug in the serum. A combination of factors seems to be responsible for this situation. Both renal function and the conjugation of chloramphenicol to its glucuronide are deficient in the newborn. This combination of deficiencies allows accumulation of the drug in the tissues, often in toxic concentrations (5, 6). For these reasons, the recommended dosage schedule to infants now consists of 50 mg. per kg. per day.

Other reactions.—Chloramphenicol is an extremely uncommon cause of angioneurotic edema, urticaria, local moniliasis, or enterocolitis, and only occasional mention is made of these reactions. Most of them are minor, but one or two fatal cases have been reported. Diarrhea and abdominal distress occur in a few patients, but the incidence is much less than with the other broad-spectrum antimicrobials. Chloramphenicol has less effect on the intestinal microflora than do the tetracyclines, perhaps because most of the dose is rapidly absorbed from the jejunum and the remainder is promptly inactivated (7).

FURALTADONE

Furaltadone is the newest of the nitrofuran derivatives to become available for general use. Its antibacterial spectrum includes many Gram-positive and Gram-negative microorganisms, and significant serum and tissue concentrations may be achieved following oral administration. The major clinical indication for furaltadone seems to rest on its effects against staphylococci

TABLE I *Continued*

Agent	Mucus Mem and Skin	G I.	Blood Marrow	C.N S.	Liver	Renal	Local	Fever	Anaphylactoid	Other	Comment	Frequency	Reference
Polymixin-B							×				Pain	F	(1)
								+				F	(1)
	+											R	(5)
Ristocetin			×							All elements		F	(1, 3 to 8)
				×						Ototoxicity		R	(1)
						×						R	(1)
	+						+	+		Often severe		F	(1, 3 to 7) (8, 9)
Streptomycin, Dihydrostreptomycin				×						VIIIth Nerve		F	(1 to 6)
	+							+				F	(8)
						×						R	(7)
						+			+			R	(7, 9)
										Encephalitis and Blindness from intrathecal use		R	(7)
New Sulfonamides	+							+				F	(3, 6)
					+ ×							R	(1, 2, 6)
		×		×	×	×				Myocarditis		R	(3 to 6)
Tetracyclines	+		+		+			+	+			R	(1 to 4)
		×										F	(1, 3, 4)
	×									Photosensitivity		R	(6, 7)
		×								Enterocolitis		R	(1, 2, 4)
										Candidiasis		F	(1, 2, 3, 5)
Vancomycin	+						×	+		Phlebitis		F	All reports
				×							Deafness	R	(1 to 5)
									+			R	(9)
						×					Chills, Shock	R	(1, 5)
			+							Eosinophilia		F	

locytosis has not been recorded to date. Thrombocytopenia has also been reported in two patients (2).

Griseofulvin

Griseofulvin is highly effective in a great variety of dermatophytoses. It must be given by mouth since local application is ineffective. Prolonged administration is necessary because of the persistence of inhibited but still viable fungi in the affected tissues. Treatment must be continued until all of the affected tissues are replaced in the normal course of tissue regeneration. This process may take six months in the case of a diseased nail and nail bed, and two to three months when tinea capitis is present. Any agent administered over such long periods of time would be expected to carry with it a significant hazard of untoward side effects but this has not been the case for griseofulvin. Only minor reactions have been described and these rarely necessitate discontinuation of therapy (1 to 8).

Toxicity.—Early studies in animals indicated that griseofulvin could act as an inhibitor of mitosis, and sperm counts were shown to be depressed in animals receiving very large doses parenterally. This has not occurred in several groups of human beings who were studied by means of serial sperm counts and testicular biopsies (1, 2, 3). Only one instance of transient azoospermia has been reported (12). The sperm count returned to normal when therapy was discontinued.

The cytotoxic effects of griseofulvin suggested that it might cause bone marrow depressions but no serious accident of this nature has been described. Several investigators reported transient fall of the total leucocyte count, which returned to normal when therapy was discontinued (1, 7, 9, 10). In some cases, therapy was continued in the face of this complication without further depression being noted.

Nausea and epigastric distress have occurred in less than 5 per cent of patients comprising the series analyzed here (1 to 9). These manifestations were more common when 2.0 gm. per day were administered and less so at the presently suggested dosage of 1.0 gm. per day. Griseofulvin is excreted very slowly and its site of activity is found in slowly metabolizing tissue sites (keratin). It may therefore be possible to reduce the dosage even further after more experience has been obtained in its use.

Headache also occurs following griseofulvin administration and may be slightly more common than intestinal upsets. It occurred in 24 of 137 patients in one series (11), and in 10 per cent of 134 patients reported elsewhere (12). It may be severe initially, but either abates spontaneously or does not recur when therapy is reinstituted after a short interruption. Aspirin may relieve this symptom.

There have been a number of other untoward reactions which could be grouped under the general category of disturbances in mood or nervous system function. Drowsiness and fatigue have occurred quite commonly.

that are resistant to the other antimicrobial agents. Its clinical value seems very limited.

Toxicity.—There is very little quantitative information available on toxic reactions to furaltadone. Nausea and vomiting may occur in one third of patients receiving this drug (1). Administration after meals, and prior grinding or dissolving of the tablets seem to bave been effective in overcoming a significant fraction of such gastrointestinal disturbances.

A very interesting phenomenon has been observed in some patients imbibing alcoholic heverages during and even several days after a course of furaltadone therapy. A combination of erythema, facial edema, bronchospasm, arthralgia, nausea, urticaria, tachypnea, hypotension, dyspnea, and a feeling of constriction of the chest has occurred in such individuals (3). This reaction is clinically very similar to that seen in patients receiving disulfiram for the control of alcoholism. To date, 60 such reactions have been reported to the manufacturer (2).

Neurological disturbances following furaltadone therapy have occurred in at least 25 instances (2). Manifestations have included diplopia with paresis of the extraocular muscles, nystagmus, blurring of vision, diminished auditory acuity, peripheral neuritis, dysphagia, slurred speech, and difficulty in phonation. Cessation of therapy was followed by complete recovery in all cases except for one diabetic patient who still complains of blurred vision.

These reactions are serious and frequent enough to have caused the manufacturer to issue three cautionary bulletins. They stress complete avoidance of alcohol in any form during and for seven days following furaltadooe therapy. Moreover, the manufacturer suggests that no therapeutic trial be extended beyond five days, and that the total duration of furaltadone therapy not exceed 14 days in an effort to minimize the neurotoxic reactions.

Furaltadone is closely related to nitrofurantoin, an agent implicated in several instances of hemolytic anemia of the primaquine-sensitive variety. A shortened survival time of primaquine-sensitive erythrocytes has heen noted (1), but no clinically recognizable hemolysis has occurred. Caution should be exercised, especially when furaltadone is administered to Negroes and patients of Mediterranean and near-eastern origin.

One patient with pre-existing kidney disease was observed who rapidly developed renal insufficiency when furaltadone was administered.

Hypersensitivity reactions.—Approximately 10 per cent of persons receiving furaltadone have developed fever and rashes. More severe reactions associated with fever, myalgia, and pruritus that could be reproduced hy readministration of the drug have been observed (1).

One-third of 24 patients in one group (1) developed eosinophilia without other signs of untoward reaction even though treatment was continued. Transient leucopenia has heen reported on two occasions. The leucocyte counts promptly returned to normal upon cessation of therapy, and agranu-

Ototoxicity was demonstrated in one study to follow the administration of 40 gm. of kanamycin and also after approximately 20 gm. of neomycin (4). Much smaller amounts of both drugs have caused this reaction in older persons and in those with renal insufficiency.

In one of the larger groups of kanamycin-treated patients (5), the incidence of severe toxicity and hypersensitivity reactions amounts to 11 per cent, or 5 of 45 patients. Three developed fatal nephrotoxicity while receiving parenteral doses of 2 gm. per day for 6, 7, and 14 days, respectively. One patient became deaf after 18 gm. given over a 9-day period, and one developed a generalized rash. These reactions are similar to those recorded in the earlier reports of neomycin toxicity.

The section on neomycin should be consulted for additional information in regard to kanamycin toxicity since all of the phenomena recorded there have been or will be experienced during the use of this new and closely related agent.

NEOMYCIN

Neomycin is a toxic agent most likely to damage the auditory nerve and the kidneys. It is poorly absorbed from the bowel and has been employed widely by mouth to suppress the gastrointestinal flora in preparation for colonic surgery and in persons with liver disease. Parenteral administration in the management of infection by staphylococci and Gram-negative bacilli resistant to the action of other antibiotics has also become established practice.

Nephrotoxicity.—Nephrotoxicity was one of the earliest documented toxic effects of neomycin (1). The lesion consists of focal areas of foamy vacuolization of the epithelial lining of the convoluted tubules, particularly the proximal segments. Proteinuria and cylindruria represent the usual clinical findings. The occurrence of nephrotoxicity is definitely related to dosage and total duration of parenteral administration. Amounts as low as 1.0 gm. per day for periods of two weeks have resulted in severe nephrotoxicity when given to patients with no known renal disease. This appears to be reversible if detected in the early stages. At least 15 fatal cases have been reported recently in which neomycin could be considered the lethal factor in patients without antecedent renal disease (2).

The potential effect of neomycin on previously damaged kidneys warrants extreme care when this agent is employed in such situations. Dosage must be adjusted to allow for delayed excretion (3).

Absorption is increased when neomycin is administered orally to patients with severe hepatic disease, ulcerative colitis, or perforation of the bowel. If there is an element of coexisting renal insufficiency, toxic blood and tissue concentrations may be attained rapidly (3, 4).

Ototoxicity.—Auditory nerve damage similar to that caused by the streptomycins but more severe and arising more rapidly, also follows par-

Vertigo, insomnia, photophobia and photosensitivity, and a feeling of excitement have also been recorded (11). These reactions are usually mild and disappear spontaneously even if therapy is continued. Only occasionally does it become necessary to discontinue treatment because of these reactions.

A possible potentiation of the effects of alcohol has been noted in a very small number of patients. This is said not to be an undesirable side effect but should be evaluated further with proper cognizance of the potential dangers involved.

One patient developed tachycardia and flushing when alcohol and griseofulvin were taken at the same time. This may be similar to the experiences with furaltadone. It occurs rarely, but may be reported more frequently in the future (9).

Transient albuminuria was noted in five patients and elevation in the icterus index in two patients in one series of 52 individuals studied (9). No other abnormalities of renal or hepatic function were noted in these or other patients. The abnormalities returned to normal when therapy was discontinued.

Hypersensitivity reactions.—Evanescent maculopapular and erythematous rashes have occurred in less than 3 per cent of the patients reported in the above studies. These reactions have usually cleared promptly when the drug was withheld. In some instances, challenges with repeated administrations of griseofulvin have clearly established its role in the etiology of these reactions. At least one case report describes a severe reaction consisting of generalized angioneurotic edema and persistent urticaria, indistinguishable from similar reactions encountered following the administration of penicillin (12). Treatment had been started and then interrupted for four months. The reaction first became manifest after two weeks of further therapy. The patient was not known to have been exposed to other drugs during this time.

Griseofulvin is elaborated by and recovered from several species of *Penicillium.* Cross-sensitivity between penicillin and griseofulvin in patients who have a history of previous penicillin allergy might therefore be expected. No such reactions have been reported to date even though the drug has already been administered to many patients with recorded prior untoward reactions to penicillin.

Kanamycin

The chemical structure and antimicrobial spectrum of kanamycin are similar to those of neomycin. Toxic reactions to the two agents are identical but present evidence indicates that kanamycin is less likely to cause deafness and renal lesions than neomycin when administered in comparable amounts. Since neomycin has a substantially greater antibacterial activity it is possible that comparison of the toxicity of the two agents on the basis of serum or tissue antimicrobial effect might demonstrate that there is no real advantage to the use of kanamycin (1 to 5).

reducing the intestinal microflora over prolonged periods of time should be sought unless rigid precautions for the early detection of nephrotoxicity, ototoxicity, and intestinal dysfunction are employed.

Hypersensitivity.—Hypersensitivity reactions to neomycin have been uncommon. Anaphylaxis has not been reported recently and dermal reactions were thought to be unusual but newer information suggests that this is not true.

Conjunctivitis.—Epstein & McCormick report instances of contact sensitization with conjunctivitis following ophthalmic instillation of neomycin (15). The combination of adrenal cortical steroids with neomycin in proprietary preparations seems to mask this reaction which then sometimes appears after therapy is discontinued. Prompt clearing of the conjunctivitis usually occurs on cessation of therapy. Intradermal tests are frequently positive but may be negative if mucous membrane sensitization has occurred in the absence of cutaneous sensitization. In the latter event, diagnosis may be established by means of instillation of neomycin solution into the eye. Prompt clearing of the lesion on withdrawal of the drug should constitute sufficient clinical proof.

Dermal contact reactions.—Epstein has emphasized the fact that neomycin is a frequent cause of contact dermatitis when applied locally (16), especially in the presence of some other form of skin disease. The eyelids are frequently involved. The delayed form of cutaneous hypersensitivity reaction may be demonstrated by intradermal tests with dilute solutions of neomycin. Neomycin-steroid combinations may mask the early manifestations of cutaneous hypersensitivity only to have these reactions present themselves in severe form when therapy is discontinued (17). Presumably, if the steroids had not been employed concomitantly, milder reactions might have been recognized earlier.

A high degree of cross-sensitization exists between neomycin and streptomycin (16, 18). Intradermal tests often confirm this but are not without danger (18). Shock and urticaria have been reported following the intradermal injection of minute amounts of both neomycin and streptomycin in highly sensitive individuals.

Nitrofurantoin

Nitrofurantoin is an antibacterial drug which has had wide acceptance in the treatment of urinary tract infections where it serves principally as a suppressive agent. Toxic and hypersensitivity reactions are very similar to those recorded for furaltadone and increase rapidly in frequency when the dose exceeds 7 mg. per kg. per day. At this dosage about 15 per cent will experience nausea and vomiting. Treatment will have to be permanently discontinued in about 10 per cent because of toxic and hypersensitivity reactions. Neither the disulfiramlike nor the neurotoxic reactions encountered with furaltadone have followed nitrofurantoin therapy.

Nitrofurantoin has been definitely identified as another in the ever grow-

enteral administration or increased absorption after oral administration (1, 5, 6). Deafness is permanent. Older persons are most susceptible. Fullness in the ears and tinnitus constitute early warning symptoms. Once these have appeared total loss of hearing may develop even though the drug is withdrawn. Patients and their attendants should be cautioned to look for and report such manifestations promptly.

Ototoxicity is limited to the cochlea and consists of atrophy or destruction of the organ of Corti (7) and degeneration of the stria vascularis and cochlear ganglion (8) in the experimental animal. The vestibular mechanisms are apparently not affected by this agent. Deafness has not followed topical application but has occurred after long-continued neomycin aerosol administration (9).

Pantothenate has been claimed to be effective in the prevention and treatment of ototoxicity due to neomycin (5). The evidence is inconclusive but this drug should be employed if deafness has appeared under therapy.

Respiratory arrest.—Respiratory arrest has followed intraperitoneal administration of neomycin at the time of abdominal surgery (10 to 12). Small children and elderly patients appear to be at high risk from this complication which has followed within minutes of the administration of 0.5 gm. of the drug in this manner. Artificial respiration has been used successfully in a few instances, but a number of fatalities have occurred.

Neomycin has been shown to be a neuromuscular blocking agent in animals. This action is enhanced by ether and antagonized by neostigmine (13). Neostigmine has been used successfully to overcome neomycin-induced respiratory arrest in patients undergoing surgery. Between 0.5 and 1.0 mg. were necessary to achieve the desired effect (11).

A drug like neomycin, with such marked stability, limited routes for excretion, and great toxicity, should be administered with extreme caution when large amounts can be absorbed rapidly. If peritoneal contamination has occurred, the use of small doses (fractions of a gram) and aspiration of the fluid before closure of the abdomen should combine to minimize this particular aspect of neomycin toxicity.

Diarrhea and steatorrhea.—Neomycin increases the number of bowel movements, rendering the stools liquid. It does not usually increase the volume of the stools or cause dehydration although some increase in mucus occurs (1). Several loose stools per day is the rule but some patients develop a profuse, watery diarrhea refractory to medical management until the drug is withdrawn (19).

Faloon and his associates have described a sprue-like syndrome with steatorrhea and significant electrolyte imbalance which developed when neomycin was given to patients with decompensated cirrhosis of the liver (14). It is of note that malabsorption did not depend on the presence of diarrhea, but also occurred when patients were constipated.

In view of these findings, and because of the increased absorption of neomycin from the intestinal tract of patients with liver disease, other means of

such patients does give the same color reactions as does biliburin. Urine tests for bile are consistently negative.

Thrombocytopenia and hemorrhagic disorders.—Novobiocin administration occasionally has been followed by thrombocytopenia and hemorrhagic disorders (7, 13, 14). Such accidents are exceedingly rare, but have been associated with lower-range dosages and have appeared as soon as the eighth day of therapy. More important, Day and his co-workers have described one instance of immunothrombocytopenia following novobiocin therapy (14), suggesting that this is a true hypersensitivity reaction.

Leucopenia and agranulocytosis.—Leucopenia has been observed on numerous occasions when the peripheral white blood count has been followed carefully (15, 16). The leucopenia has subsided spontaneously with or without discontinuation of therapy, and appears to be a relatively minor complication. Agranulocytosis has been reported on one occasion (15) but the clinical picture was obscured by a number of other potential agranulocytosis-producing agents to which the patient had also been exposed. This untoward reaction is certainly extremely rare if it is ever caused by novobiocin.

Other untoward reactions.—Transient dizziness, and blurring of vision have occasionally been reported to follow immediately upon oral administration of novobiocin (17, 18). In at least one instance, the patient was markedly uremic before he experienced this reaction. There is no clinical or experimental evidence to suggest true neurotoxicity from novobiocin unless large doses are applied directly to the cortex of experimental animals (5).

Staphylococcal enteritis followed by monilia enterocolitis has been reported with novobiocin administration in one instance (19). While rare, this occurrence emphasizes the fact that any interference with the intestinal microflora may result in this dreaded and often lethal complication.

One instance of an allergic cutaneous reaction resembling the Stevens-Johnson syndrome has also been described (5). This case was associated with marked nuchal rigidity, irritability, stupor, and pleiocytosis in the spinal fluid with negative bacteriological and viral studies. This case is unexplained.

Drug fever.—Most investigators and clinicians have been struck by the occasional occurrence of very high, sustained fever occurring during novobiocin therapy. The exact incidence of this reaction is difficult to determine but exceeded 10 per cent in several groups of cases when 2.0 gm. per day or more were administered (1, 7). The frequency of this reaction at lower dosages is not so well defined, but fever attributable to novobiocin is one of its more common side reactions.

RISTOCETIN

Ristocetin is an antimicrobial agent of value in the treatment of infection by staphylococci and other Gram-positive organisms resistant to previously available agents. Use of this drug will be limited since it must be given intra-

ing list of substances capable of causing hemolysis in susceptible individuals (1, 2).

Under the broad classification of primaquine-sensitive hemolytic anemias, hemolysis has been shown to result from an enzymatic defect in the erythrocyte when the cells of susceptible individuals are exposed to any one of a number of drugs and chemicals. This inherited abnormality is particularly common among Negro males. It also occurs among ethnic groups of Mediterranean and near-eastern origin. Nitrofurantoin should be used with caution in such patients.

Novobiocin

Untoward reactions to novobiocin are common. Most prominent are rashes and gastrointestinal upsets.

Cutaneous reactions.—Cutaneous reactions to novobiocin usually appear as maculopapular eruptions within seven to ten days after commencement of therapy. Morbilliform eruptions, urticaria, severe pruritus, and angioneurotic edema have also been described (1 to 9). In collected series totaling over 700 patients, the average incidence of cutaneous reactions exceeds 10 per cent, the distribution ranging from none in 30 patients to severe reactions associated with high fever in 24 of 45 patients. There is very little doubt that the amount and duration of drug administration significantly affects the incidence of cutaneous reactions. The frequency of untoward reactions rises sharply when a dose of 1.0 gm. per day is exceeded, or when the course of therapy exceeds one week.

Gastrointestinal tract.—The incidence of nausea, vomiting, abdominal discomfort, and diarrhea also seems to be related to the dosage. At the lower range, the incidence of gastrointestinal reactions approximates 10 per cent; most are minor and therapy need not be discontinued. At higher dosages, the incidence of reactions may approximate 50 per cent, but most of these are also mild. Only occasionally does intractable vomiting or diarrhea require cessation of therapy (1 to 9). It is generally believed that these side effects can be minimized if the drug is given on a full stomach.

Novobiocin "jaundice."—Yellow discoloration of the skin, sclerae, and serum has been observed sporadically ever since novobiocin was first released (10, 11, 12). It has usually been associated with dosages greater than 2.0 gm. per day but has occasionally followed considerably smaller amounts. Children appear to be especially susceptible to this complication. A rough approximation of the reported incidence of "jaundice" places it at 1 per cent. It has been claimed that the discoloration represents unconjugated bilirubin, and that novobiocin is therefore a hepatotoxin (10). The evidence for this is slight. Therapy has been continued, liver function tests have remained normal, and the discoloration has disappeared spontaneously in a number of instances. The discoloration is probably caused by a chromogenic metabolite of novobiocin, although this substance has not been identified. The serum of

with ristocetin (5, 9). It is believed to be less common with the newer and more highly purified preparations of the drug currently employed. Fever usually subsided promptly when therapy was discontinued.

Thrombophlebitis and irritation following the local infiltration of ristocetin occurred relatively frequently when this agent was first employed. More recently, the use of small bore needles, the frequent rotation of injection sites, the use of intermittent rather than continuous injections, and the further purification of the agent itself seem to make these complications less troublesome.

It has been recently suggested that the usual daily dose of ristocetin may be administered intramuscularly to infants and to patients for whom intravenous injection is particularly difficult (10). The addition of 1.0 mg. of hydrocortisone for each 100 mg. of ristocetin in the injection appeared to diminish the local irritation sufficiently for this form of therapy to be tolerable. The recommended intramuscular dose for infants was the same as the recommended intravenous dose, or 50 mg. per kg. of body weight per 24-hr. period, administered in two injections per day.

An interesting relationship has been established between the amount of administered ristocetin and the incidence of untoward effects (5). At a range of dosage from .1 to .9 gm. per day, there was a reaction rate of 3.1 per cent; at 1.0 to 1.9 gm. per day, it was 9.4 per cent; at 2.0 to 2.9 gm. per day, it was 21.2 per cent; and at 4.0 to 4.9 gm. per day, it was 41.0 per cent.

The Streptomycins

Hypersensitivity and toxic reactions consisting of skin rash, fever, and damage to the eighth nerve are the main side effects of the streptomycins.

The last has attracted much attention recently since total permanent deafness has frequently followed the administration of small amounts of dihydrostreptomycin (1, 2). In certain cases this disaster occurred after only a few grams of the drug had been given (3). Streptomycin may cause vestibular damage but an adjustment to the associated vertigo usually can be made by most younger patients. Elderly patients should receive streptomycin only when the indications for its use are clear.

The ototoxic dose of these two agents is not precisely known but deafness or severe vertigo, or both, are known to have followed the administration of 2 to 20 gm. (3 to 6). The onset of deafness may be delayed by several weeks after withdrawal of dihydrostreptomycin (3) but streptomycin neurotoxicity always appears during therapy.

Combinations of streptomycin and dihydrostreptomycin have been evaluated (6). Deterioration of hearing as demonstrated by audiographic study occurred three times as frequently with the combination as with streptomycin alone in equivalent dosage. Dihydrostreptomycin clearly is too toxic for clinical use since a safer drug, streptomycin, is available. Dihydro-

venously, may produce serious toxic and hypersensitivity reactions, and its clinical effectiveness has not been fully established (1, 2).

Toxicity.—One of the most serious reactions to ristocetin is thrombocytopenia which is due to a direct toxic effect upon the platelets in the circulating blood (3). Megakaryocytes are present in the bone marrow and active platelet production occurs even during episodes of platelet lysis. Ristocetin has been shown to cause disintegration of platelets when added to a platelet suspension or normal blood. Thrombocytopenia and other hematological side effects seem to be closely related to dosage. Eight of ten patients receiving 50 mg. per kg. per day of ristocetin developed such reactions (4), whereas the incidence was much lower in patients receiving only 25 mg. per kg. per day. Thrombocytopenia occurred in 1.5 per cent of 333 collected cases treated with ristocetin and reported by the manufacturer (5).

Agranulocytosis, leucopenia, and neutropenia have also followed ristocetin administration. The incidence of these reactions seems closely related to dosage and length of therapy, and was approximately two to three times that of thrombocytopenia (1, 4 to 8). It is not clear whether these reactions are purely toxic in oature or whether there is an associated element of hypersensitivity. Many of the patients who experienced depression of the leucocytic elemeots also had ao associated eosinopbilia and fever (4, 6, 8). Instances of leucopenia have occurred after days or weeks following cessation of therapy, possibly indicating an element of delayed hypersensitivity (3).

Anemia is a rare complicatioo of ristocetin therapy, appearing suddenly and occasionally associated with overt bleeding (3, 4). Unrecognized blood loss in patients with thrombocytopenia may have explained its presence in other cases, but rouleaux formation and hemolysis have been demonstrated *in vitro* wheo erythrocytes were exposed to concentrations of ristocetin causing platelet agglutinatioo and lysis (3).

One of 51 patients reported in one treatment series (1) developed progressive, irreversible deafness which began 24 hr. after the institution of ristocetin therapy. Its role in the patbogenesis of this complication could not be evaluated since this individnal had also received neomycin and vancomycin.

The nephrotoxicity of ristocetio is slight aod only one instance of renal failure associated with its use has beeo recorded (1). Excessive serum and tissue concentrations may be anticipated and the dosage reduced accordingly when the drug is administered to patients with kidney disease.

Hypersensitivity reactions.—Between 4 aod 20 per cent of patients treated with ristocetin have developed rashes which usually disappeared when the drug was withdrawn (1, 4, 5, 9, 10). Two disturbing reports describe exfoliative dermatitis in one-third of those individuals who exhibited any dermal lesion (1, 4). Confirmation of this observation would be further indication for great caution in the use of ristocetio.

Drug fever has occurred in approximately 5 per cent of patients treated

Toxicity.—Ototoxicity is the major hazard of vancomycin therapy (1 to 5). This effect does not seem to be related to dosage or duration of therapy except insofar as these are reflected in excessively high serum concentrations. Such high concentrations may result when the recommended daily dosages of 2.0 gm. to adults, and 25 to 60 mg. per kg. to infants and children, are exceeded, or if the drug is given to patients with impaired renal function without reductions in dosage (4). When vancomycin is employed in the presence of renal insufficiency, serial studies of renal function and audiometry should be obtained. The drug should be discontinued at the earliest symptom or sign of ototoxicity, otherwise deafness will be complete and irreversible.

Nephrotoxicity with albuminuria and progressive azotemia was described in earlier reports but has been less of a problem with the more recent preparations. In accumulated series comprising approximately 100 patients, no evidence of renal impairment was recorded (2, 5 to 8). Three of 54 patients in one group developed severe azotemia with a fatal termination in one (1). Serial determinations of the nonprotein nitrogen concentration in the serum and of the urinary sediment are desirable in all instances in which vancomycin is employed.

Phlebitis at the site of intravenous injection was and still is a very common occurrence. It appeared in every patient treated in some series (7), and was noted in approximately 20 to 25 per cent of the patients in others (5, 6, 8). Kirby states that continuous infusions are much more hazardous in this respect than when the following regimen is adopted (5). Vancomycin is diluted in the ampoule as directed, and administered through the tubing of an intravenous infusion which had been started earlier, taking 5 to 10 min. for the injection. The calculated daily dose is divided into four equal fractions and injected on a six-hourly schedule. Small bore, scalp vein needles in the peripheral veins or a catheter placed in one of the larger veins are used. The infusion site is rotated at least every 24 hr. unless a catheter is *in situ*.

Severe chills, fever, and vascular collapse occur unpredictably when vancomycin is injected intravenously (1, 5), but these reactions are usually not prolonged and are often controllable with antihistaminic medications. They were noted frequently when vancomycin was first made available, but their occurrence today can be estimated to affect no more than 1 or 2 per cent of patients to whom this agent is properly administered.

Hypersensitivity reactions.—Only one case of a non-fatal anaphylactoid reaction has been described to date (9). This occurred in an individual who also manifested marked hypersensitivity to novobiocin and sulfonamides.

Cutaneous reactions consisting of generalized rashes similar to those encountered with ristocetin have been noted in almost every series reported (1, 5, 6). These rashes have been urticarial, erythematous, or maculopapular alone or in combination. Their incidence in vancomycin-treated patients is somewhere around 5 per cent. Most of these are mild but occasionally progress when therapy is continued, and may finally necessitate discontinuation

streptomycin has now been withdrawn from all combinations containing penicillin and is available only in mixtures with streptomycin. The use of the latter preparations should be avoided.

Streptomycin may be administered in a dose of 1.0 gm. per day for two to three weeks without great risk if renal function is normal. The drug will accumulate rapidly if excretion is impaired and serious toxicity may develop even though the daily dose is small.

The Tetracyclines

Toxic and hypersensitivity reactions are virtually unknown in association with the administration of tetracycline, oxytetracycline, and chlortetracycline (1 to 4). Gastrointestinal disturbances, including anorexia, nausea, vomiting, and diarrhea, are relatively common. Two grams per day or more may cause such reactions in 10 per cent of patients but smaller amounts are much less likely to do so (1, 3, 4). Glossitis, stomatitis, and pruritus ani and vulvae are also frequently observed (1, 3). Some investigators believe that these disorders are caused by overgrowth of *Candida* spp. in the affected areas but this has not been proved. The concurrent administration of an antifungal agent has been claimed to reduce the incidence of this type of reaction (5).

The most serious untoward effects of tetracycline therapy are those attributed to the suppression of the normal bacterial flora of the external and internal surfaces of the body, which permits overgrowth and superinfection by various potentially pathogenic microorganisms. This interesting and complex subject is beyond the scope of this review.

Recently a new agent, demethylchlortetracycline, has been introduced. It is well absorbed and more slowly excreted than earlier tetracyclines so that a smaller total daily dose may be effective.

Untoward effects associated with its use are similar to those experienced with the other tetracyclines with one exception. Photosensitivity has been described following the use of demethylchlortetracycline (6, 7). Limited exposure to sunlight was followed by the rapid onset of erythema and swelling in five patients. These reactions were later reproduced by readministration of the drug under similar circumstances. Patients receiving this agent should protect themselves from the sun. On the basis of our own experience we believe that this reaction occurs much more commonly in exposed patients than the literature available to date would indicate.

Vancomycin

Vancomycin is one of the more recent and most potent additions to the antistaphylococcal armamentarium. When properly used, vancomycin is essentially safe, although coexisting renal damage significantly increases the hazards of toxic reactions. Its chief disadvantage is that it must be administered intravenously and that phlebitis occurs rather commonly.

LITERATURE CITED

GENERAL REVIEWS

1. Carr, E. A., *New Engl. J. Med.*, 245, 829–900 (1951)
2. Carr, E. A., *New Engl. J. Med.*, 245, 935–40 (1951)
3. Meyler, L., *Side Effects of Drugs* (Elsevier Publ. Co., Inc., New York, N. Y., 1952)
4. Hall, W. H., *Minnesota Med.*, 35, 625 (1952)
5. Finland, M., and Weinstein, L., *New Engl. J. Med.*, 248, 220–26 (1953)
6. von Oettigen, W. F., *Am. J. Med.*, 18, 792–809 (1955)
7. Lowell, F. C., *Ann. Internal Med.*, 43, 333–44 (1955)
8. Alexander, H. L., *Reactions to Drug Therapy* (W. B. Saunders Co., Philadelphia, Pa., 1955)
9. *Pediatric Clinics of North America*, 3 (Symposium on Antimicrobial Therapy, 1956)
10. Welch, H., Lewis, C. N., Weinstein, H. I., and Boeckman, B. B., *Antibiotic Med. & Clin. Therapy*, 4, 800–13 (1957)
11. *Brit. Med.. Bull.*, 16, 1–88 (1960)

AMPHOTERICIN-B

1. Seabury, J. H., and Dascomb, H. E., *Arch. Internal Med.*, 102, 960–76 (1958)
2. Utz, J. P., Treger, A., McCullough, M. B., and Emmons, C. W., *Antibiotics Ann., 1958/59*, 628–34 (1959)
3. Winn, W. A., *Am. J. Med.*, 27, 617 (1959)
4. Newcomer, V. D., Sternberg, T. H., Wright, E. T., and Reisner, R. M., *J. Chronic Diseases*, 9, 353–74 (1959)
5. Hunter, R. C., and Mongan, E. S., *U. S. Armed Forces Med. J.*, 9, 1474–86 (1958)
6. Littman, M. L., Horowitz, P. L., and Swadey, J. G., *Am. J. Med.*, 24, 568–92 (1958)
7. Shields, L. H., *Arch. Internal Med.*, 104, 763 (1959)
8. Sarot, I. A., Littman, M. L., and Cerruti, M. M., *Sea View Hosp. Bull.*, 17, 95–100 (1959)
9. Costello, M. J., DeFeo, C. P., Jr., and Littman, M. L., *Arch. Dermatol.*, 79, 184–93 (1959)

BACITRACIN

1. Meleney, F. L., *Practitioner*, 176, 56 (1956)

CHLORAMPHENICOL

1. Welch, H., Lewis, C. N., and Kerlan, I., *Antibiotics & Chemotherapy*, 4, 607–23 (1954)
2. Welch, H., Lewis, C. N., Weinstein, H. I., and Boeckman, B. B., *Antibiotic Med. & Clin. Therapy*, 4, 800–13 (1957)
3. Report to the Council on Drugs, *J. Am. Med. Assoc.*, 172, 2044–45 (1960)
4. Dunlop, D. M., and Murdoch, J. McC., *Brit. Med. Bull.*, 16, 68–69 (1960)
5. Burns, I. E., Hodgman, J. E., and Cass, A. B., *New Engl. J. Med.*, 261, 1318–21 (1959)
6. Weiss, C. F., Glazko, A. J., and Weston, J. K., *New Engl. J. Med.*, 262, 787–94 (1960)
7. Woodward, T. E., and Wisseman, C. L., Jr., "Chloromycetin," *Antibiotic Monographs, No. 8*, 25 (Medical Encyclopedia, Inc., New York, N. Y., 1958)

ERYTHROMYCIN-OLEANDOMYCIN-SPIRAMYCIN

1. Dunlop, D. M., and Murdoch, J. McC., *Brit. Med. Bull.*, 16, 67–72 (1960)
2. Forfar, J. O., and Maccabe, A. F., *Antibiot. et Chemotherap.* (Basel), 4, 115–57 (1957)
3. Welch, H., Lewis, C. N., Weinstein, H. I., and Boeckman, B. B., *Antibiotic Med. & Clin. Therapy*, 4, 800–13 (1957)
4. Stryker, H., Siegel, B. B., and Grolnick, M., *Antibiotic Med. & Clin. Therapy*, 5, 723–25 (1958)
5. Solomon, S., and Johnstone, B., *Am. J. Med. Sci.*, 230, 660–74 (1955)
6. Romansky, M. J., Nasou, J. P., Davis, D. S., and Ritts, R. E., *J. Am. Med. Assoc.*, 164, 1197–1204 (1957)
7. Bower, A. F., *California Med.*, 89, 279–80 (1958)
8. Spink, W. W., *Arch. Internal Med.*, 94, 167 (1954)
9. Blough, H. A., Hall, W. H., and Hong, L., *Am. J. Med. Sci.*, 239, 539–47 (1960)

FURALTADONE

1. McCabe, W. R., Jackson, G. G., and Kozij, V. M., *Antibiotics Ann. 1959–60*, 776–84 (1960)
2. McLeod, P. F. (Personal communication)
3. Loftus, L. R., and Wagner, A. W., *J. Am. Med. Assoc.*, 173, 362–63 (1960)

of the planned regimen. Contrary to the experience with ristocetin, exfoliation is extremely uncommon and has not been reported recently.

Drug fever occurs with or without other manifestations of hypersensitivity or toxicity in 2 or 3 per cent of patients receiving vancomycin. The temperature elevations are not great and fever almost never requires cessation of therapy.

Eosinophilia has also occurred in most reported studies, but no other changes in the bone marrow or peripheral blood have been noted.

2. Anderson, J. R., and Rubin, W., *Eye, Ear, Nose, Throat Monthly*, 38, 638–41 (1959)
3. Herrold, R. D., *J. Urol.*, 77, 771–72 (1957)
4. Hughes, J., *Antibiotics Med.*, 5, 559–61 (1938)
5. Hughes, J. G., *J. Pediat.*, 51, 664–66 (1957)
6. Pulaski, E. J., and Isokane, R. K., *Surg. Gynecol. Obstet.*, 104, 310–18 (1957)
7. Reig, A., and Lopez, A., *Lancet*, II, 972–73 (1959)
8. Ward, V., and Meyer, D. O., *Wisconsin Med. J.*, 56, 127–32 (1957)
9. Herrold, R. D., *J. Urol.*, 79, 1010–13 (1958)
10. Cox, R. D., Folz, E. L., Raymond, S., and Drewyer, R., *New Engl. J. Med.*, 261, 139–41 (1959)
11. Welch, H., Lewis, C. N., Putnam, L. E., and Randall, W. A., *Antibiotic Med. & Clin. Therapy*, 3, 27–32 (1956)
12. Huerta-Carlock, A., *Gac. Méd. (Ecuador)*, 13, 434–35 (1958)
13. Breese, B. B., Disney, F. A., and Talpey, W. B., *Antibiotic Med.*, 4, 347–51 (1957)
14. Day, H. J., Conrad, F. G., and Moore, J. E., *Am. J. Med. Sci.*, 236, 475–82 (1958)
15. Simon, H. J., and Rogers, D. E., *Ann. Internal Med.*, 46, 778–84 (1957)
16. Pearson, J. Z., Somberg, A., Rosenthal, I., Lepper, M. H., Jackson, G. G., and Dowling, H. F., *Arch. Internal Med.*, 98, 273–83 (1956)
17. Kirby, W. M. M., Hudson, D. G., and Noyes, W. D., *Arch. Internal Med.*, 98, 1–7 (1956)
18. Brown, R., Thomassen, P. R., and Singler, J. M., *Oral Surg., Oral Med., Oral Pathol.*, 11, 598–602 (1958)
19. Baden, W. F., *Am. J. Obstet. Gynecol.*, 74, 47–52 (1957)

PENICILLIN

1. Smith, L. W., and Walker, A. D., *Penicillin Decade* (Arundel Press, Washington, D. C., 1951)
2. Welch, H., Lewis, C. N., Weinstein, H. I., and Boeckman, B. B., *Antibiotic Med. & Clin. Therapy*, 4, 800–13 (1957)
3. Smith, V. M., *New Engl. J. Med.*, 257, 447–51 (1957)
4. Annotations, *Lancet*, I, 688 (1960)
5. Maganzini, H. C., *New Engl. J. Med.*, 256, 52–56 (1957)
6. Paull, A. M., *New Engl. J. Med.*, 252, 128–29 (1955)
7. Symmers, W. St. C., in Rosenheim, M. L., and Moulton, R., Eds., *Sensitivity Organizations of Medical Sciences, Established under the Joint Auspices of UNESCO and WHO* (Blackwell Sci. Pubs., Oxford, Engl., 1958)
8. Dunlop, D. M., and Murdoch, J. McC., *Brit. Med. Bull.*, 16, 67–72 (1960)

POLYMIXIN-B

1. Swift, P. N., and Bushby, J. R. M., *Lancet*, I, 110 (1953)
2. Kagan, B. M., Krevsky, D., Milzer, A., and Locke, M., *J. Lab. Clin. Med.*, 37, 402 (1951)
3. Jawetz, E., *Arch. Internal Med.*, 89–90 (1952)
4. Hopper, J., Jawetz, E., and Hinman, F., *Ann. J. Med. Sci.*, 225, 402 (1953)
5. Jawetz, E., "Polymixin, Neomycin Bacitracin," *Antibiotic Monographs, No. 1* (Medical Encyclopedia, Inc., New York, N. Y., 1956)

RISTOCETIN

1. Walsbren, B. A., Kleinerman, L., Skemp, J., and Bratcher, G., *Antibiotics Ann. 1959/60*, 497–515 (1960)
2. Rantz, L. A., and Jawetz, E., *New Engl. J. Med.*, 259, 963–66 (1958)
3. Gangarosa, E. J., *Antibiotics Ann. 1959/60*, 536–48 (1960)
4. Gangarosa, E. J., Landerman, N. S., Rosch, P. J., and Herndon, E. G., *New Engl. J. Med.*, 259, 156 (1958)
5. Herting, R. L., Lees, B., Zimmerman, A. J., and Berryman, G. H., *J. Am. Med. Assoc.*, 170, 176–79 (1959)
6. Koch, R., Dries, C. P., and Asay, L. D., *Antibiotics Ann. 1959/60*, 917–21 (1960)
7. Calvy, G. L., and Schumacher, L R., *J. Am. Med. Assoc.*, 167, 1584–86 (1958)
8. Newton, R. M., and Ward, V. G., *J. Am. Med. Assoc.*, 166, 1956–59 (1958)
9. Billow, B. W., Martorella, F. J., Lupini, B., and Paley, S. S., *Antibiotics Ann. 1958/59*, 447–53 (1959)
10. Dries, C. P., and Koch, R., *J. Diseases Children*, 99, 752–56 (1960)

STREPTOMYCIN-DIHYDROSTREPTOMYCIN

1. Editorial, *New Engl. J. Med.*, 261, 666–67 (1959)

GRISEOFULVIN

1. Goldman, L., Schwartz, J., Preson, R. H., Beyer, A., and Loutzenheiser, J., *J. Am. Med. Assoc.*, 172, 532–37 (1960)
2. Blank, H., Smith, G., Jr., Roth, F. J., and Zaias, N., *J. Am. Med. Assoc.*, 171, 2168–73 (1959)
3. McCuistion, C. H., Jr., Lawlis, M. G., and Gonzales, B. B., *J. Am. Med. Assoc.*, 171, 2174–80 (1959)
4. Russel, B., Frain-Bell, W., Stevenson, C. J., Riddell, R. W., Djavahiszwili, N., and Morrison, S. L., *Lancet*, I, 1141–47 (1960)
5. Cochrane, T., and Tullett, A., *Brit. Med. J.*, II, 286–87 (1959)
6. Beare, M., and McKenzie, D., *Brit. Med. J.*, II, 1137–40 (1959)
7. Barlow, A. J. E., Chattaway, F. W., Hargreaves, G. K., and La Touche, C. J., *Brit. Med. J.*, II, 1141–43 (1959)
8. Editorial, *Brit. Med. J.*, II, 1165 (1959)
9. Robinson, M., *Antibiotics Ann. 1959/60*, 680–86 (1960)
10. Frank, L., Steiner, K., Kaufman, J., and Chiaramonte, J., *N. Y. State J. Med*, 60, 1230–33 (1960)
11. Goldblatt, S, *J. Am. Med. Assoc.*, 172, 1643–44 (1960)
12. Goldfarb, N. J., Rosenthal, S. A., Back, A., Borota, A., Dick, L., Furnari, D., and Fraser, C., *Antibiotics Ann. 1959–60*, 693–700 (1960)

KANAMYCIN

1. Lecca, G. G., Terry, J., Maggiolo, L., and Morales, A., *J. Am. Med. Assoc.*, 170, 2064–68 (1959)
2. Berman, L. B., and Katz, S., *Ann. N. Y. Acad. Sci.*, 76, 149–56 (1958)
3. Hawkins, J. E., Jr., *Ann. Otol. Rhinol. & Laryngol.*, 68, 698–715 (1959)
4. Organick, A. B., *Am. Rev. Resp. Diseases*, 81, 256–58 (1960)
5. Waisbren, B. A., Kleinerman, L., Skemp, J., and Bartcher, G, *Antibiotics Ann. 1959/60*, 497–515 (1960)

NEOMYCIN

1. Waksman, S. A., *Neomycin* (Rutgers University Press, New Brunswick, N. J., 1953)
2. Powell, L., and Hooker, J. W., *J. Am. Med. Assoc.*, 160, 557–60 (1956)
3. Kunin, C., Chalmers, T. C., Leevy, C. M., Sebastyen, S. C., Lieber, C. S., and Finland, M., *New Engl. J. Med.*, 262, 380–85 (1960)
4. Last, P. M., and Sherlock, S., *New Engl. J. Med.*, 262, 385–89 (1960)
5. Greenwood, G. J., *Arch. Otolaryngol.*, 69, 390–97 (1959)
6. Koertge-Stoeppler, S., and Mittag, G., *Münch. med. wochschr.*, 100, 1189–92 (1958) (Abstr. *J. Am. Med. Assoc.*, 168, 1285, 1958)
7. Hawkins, J. E., Jr., and Lurie, M. H., *Ann. Otol. Rhinol. & Laryngol.*, 62, 1128 (1953)
8. Riiskaer, N., Christensen, E., Petersen, P. V., and Weidman, H., *Acta Oto-Laryngol.*, 46, 137 (1956)
9. Fuller, A., *Lancet*, I, 1026 (1960)
10. Doremus, W. P., *Ann Surg.*, 149, 546–48 (1959)
11. Middleton, W. H., Morgan, D. D., and Moyers, J., *J. Am. Med. Assoc.*, 165, 2186–87 (1957)
12. Webber, B. M., *Arch. Surg.*, 75, 174–76 (1957)
13. Pittinger, C. B., and Long, J. P., *Antibiotics & Chemotherapy*, 8, 198–203 (1958)
14. Jacobsen, E. D., Chodos, R. B., and Faloon, W. W., *Am. J. Med.*, 28, 524–33 (1960)
15. Epstein, S, and McCormick, G. L., *Arch. Ophthalmol. (Chicago)*, 60, 1000–2 (1958)
16. Epstein, S, *Ann. Allergy*, 16, 268–80 (1958)
17. Goldberg, L. C., *Antibiotic Med. & Clin. Therapy*, 4, 795–96 (1957)
18. Sidi, E., Hincky, M., and Longueville, R., *J. Invest. Dermatol.*, 30, 225–31 (1958)
19. Dawson, A. M., McLaren, J., and Sherlock, S, *Lancet*, II 1263–68 (1957)

NITROFURANTOIN

1. Alving, A. S., Kellermeyer, R. W., Tarlov, A., Schrier, S., and Carson, P. E., *Ann. Internal Med.*, 49, 240–47 (1958)
2. Kimbro, E. L., Jr., Sachs, M. V., and Torbert, J. V., *Bull. Johns Hopkins Hosp.*, 101, 245 (1957)
3. Trafton, H. W., Beutner, E. H., Petronio, J. J., Lind, H. E., and Correia-Branco, M., *New Engl. J. Med*, 252, 383–87 (1955)
4. Jawetz, E., Hopper, J., and Smith, D. R., *Arch. Internal Med.*, 100, 549–57 (1957)

NOVOBIOCIN

1. Bridges, R. A., Berendes, H., and Good, R. A., *J. Pediat.*, 50, 579–85 (1957)

ORAL HYPOGLYCEMIC AGENTS[1]

By Alexander Marble, *M. D.*

Department of Medicine, Harvard Medical School; Joslin Clinic and New England Deaconess Hospital, Boston, Massachusetts

The introduction of carbutamide (1-butyl-3-*p*-aminobenzene sulfonylurea) (BZ-55; Invenol, Nadisan) into clioical use in Germany in 1955 (1, 2) quickly aroused widespread interest in oral hypoglycemic agents. This led to ao extraordinary amount of activity throughout the world botb in research laboratories and in clinics, resulting in a huge volume of publications during the past five years. Because of the difficulty soon encountered in elucidating the mode and site of action of the oral agents, the attention of investigators was turned anew to the nature of the underlying defect in diabetes and the method and place of action of insulin, still not well understood. In succession, certain agents were given clinical trial with large numbers of patients. Following closely on the heels of carbutamide came tolbutamide (1-butyl-3-*p*-toluene sulfonylurea) (D860; Orinase, Artosin, Dolipol, Rastinon). Then chlorpropamide (1-propyl-3-*p*-chlorobenzene sulfonylurea) (Diabinese), and later metahexamide (WP 40) (1-cyclohexyl-3-[*m*-amino-*p*-methylbenzene sulfonylurea]), were introduced. In addition to these closely related arylsulfonylureas, a totally different group of synthetic chemical compounds, the biguanides, was subjected to study in animals and to clinical trials in patients. Of the biguanides, the ones used most extensively have been phenformin (phenethylbiguanide) (W32; DBI) and butyl-biguanide (W37; DBV). In almost unprecedented number and frequency from 1956 until the fall of 1959, symposia were held at which laboratory workers and clinicians presented data resulting from studies regarding basic problems in diabetes and the oral hypoglycemic compounds. In most instances, papers presented at these symposia were published together. The reader may find conveniently a large part of the relevant literature by consulting issues of journals containing groups of articles regarding the oral hypoglycemic compounds (3 to 13). In addition, the complete review by Duncan & Baird (14), in which 451 references are listed, should be consulted.

During the year just preceding the final work on this review, the furious pace of research in the field or oral hypoglycemic agents slackened considerably. Investigators in the laboratory were slowed down by the difficulty in advancing beyond the point of understanding that had been reached. Clinicians were busily engaged in acquiring more extensive experience with the various agents available. New compounds introduced were few and, as of the present writing, have not secured an established place in clinical use. Three years before, in the fall of 1957, carbutamide had been withdrawn from fur-

[1] Review of the literature was, in general, terminated with the August 1960 issue of the journals to which reference is made.

2. Editorial, *Brit. Med. J.*, I, 1036–37 (1960)
3. Shambaugh, G. E., Jr., Derlacki, E. L., Harrison, W. H., House, H., House, W., Hildyard, V., Schuknecht, H., and Shea, J. J., *J. Am. Med. Assoc.*, 170, 1657–60 (1959)
4. Penman, A. C., Dickson, I., and Miller, J. S., *Tubercle*, 38, 422–24 (1957)
5. Cawthorne, T., and Ranger, D., *Brit. Med. J.*, I, 1444–47 (1957)
6. Wier, J. A., Storey, P. B., Curry, F. J., and Schless, J. M., *Diseases of Chest*, 30, 628–32 (1956)
7. Dunlop, D. M., and Murdoch, J. McC., *Brit. Med. Bull.*, 16, 67–72 (1960)
8. Wilson, H. T., *Brit. Med. J.*, I, 1378–81 (1958)
9. Welch, H., Lewis, C. N., Weinstein, H. I., and Boeckman, B. B., *Antibiotic Med. & Clin. Therapy*, 4, 800–13 (1957)

NEWER SULFONAMIDES

1. Scott, J. L., Cartwright, G. E., and Wintrobe, M. M., *Medicine*, 38, 119–72 (1959)
2. Welch, H., Lewis, C. N., and Kerlan, I., *Antibiotics & Chemotherapy*, 4, 607–23 (1954)
3. Lehr, D., *Ann. N. Y. Acad. Sci.*, 69, 417–47 (1957)
4. Tisdale, W. A., *New Engl. J. Med.*, 258, 687–90 (1958)
5. Janovsky, R. C., *J. Am. Med. Assoc.*, 172, 155–57 (1960)
6. Compilation of Data from the Pertinent World Literature—Courtesy of Lederle Laboratories (1959)

TETRACYCLINES

1. Dunlop, D. M., and Murdoch, J. McC., *Brit. Med. Bull.*, 16, 67–73 (1960)
2. Welch, H., Lewis, C. N., Weinstein, H. I., and Boeckman, B. B., *Antibiotic Med. & Clin. Therapy*, 4, 800–13 (1957)
3. Dowling, H. F., "Tetracycline," *Antibiotic Monographs, No. 3*, (Medical Enclopedia, Inc., New York, N. Y., 1955)
4. Newman, C. R., *Ann. Internal Med.*, 45, 409–44 (1956)
5. Stone, M. L., and Mersheimer, W. L., *Antibiotics Ann. 1955/56*, 862–66 (1956)
6. Morris, W. E., *J. Am. Med. Assoc.*, 172, 1155–56 (1960)
7. Falk, M. S., *J. Am. Med. Assoc.*, 172, 1156–57 (1960)

VANCOMYCIN

1. Waisbren, B. A., Kleinerman, L., Skemp, J., and Bratcher, G., *Antibiotics Ann. 1959/60*, 497–515 (1960)
2. Geraci, J. E., Heilman, F. R., Nichols, D. R., and Wellman, W. E., *Proc. Staff Meetings Mayo Clinic*, 33, 172–81 (1958)
3. Dutton, A. A. C., and Elmes, P. C., *Brit. Med. J.*, I, 1144–49 (1959)
4. Wallace, I. R., and Carson, N. A. J., *Lancet*, I, 519–20 (1960)
5. Kirby, W. M. M., Perry, D. M., and Bauer, A. W., *New Engl. J. Med.*, 262, 49–55 (1960)
6. Spears, R. L., and Koch, R., *Antibiotics Ann. 1959/60*, 798–803 (1960)
7. Geraci, J. E., and Heilman, F. R., *Proc. Staff Meetings Mayo Clinic*, 35, 316–25 (1960)
8. Riley, H. D., and Ryan, N. J., *Antibiotics Ann. 1959/60*, 908–916 (1960)
9. Rothenberg, H. J., *J. Am. Med. Assoc.*, 171, 1101–2 (1959)

doses of 500 mg. of metahexamide were given to each of four subjects, the average half life was 19.6 hr.

The sulfonylureas are eliminated from the body only by the kidneys. Fortunately, the drugs or their derivatives are readily soluble in urine so that crystalluria has not complicated treatment with these compounds.

Mode and site of action.—In his early work with hypoglycemic sulfonamides in the period of 1942–1946, Loubatières (25, 26) proposed as a working hypothesis that the lowering of the blood sugar was caused by a direct stimulation of the insulin-secreting cells of the pancreas. Despite considerable controversy and disagreement during the months which followed the announcement in 1955 of the hypoglycemic effect in human subjects with diabetes, the concept of Loubatières won much acceptance from the start. As time has passed, more and more data have accumulated to indicate that the chief site of action of the sulfonylurea compounds is in the pancreas, the drugs stimulating the secretion or release of insulin, or both. General support for this idea is found in the facts that the sulfonylureas are not effective in depancreatized men or animals (27, 28) [except, to some extent, in the latter if insulin is administered concomitantly (29)], and that the diabetic patients who respond best are those in whom the evidence is strong that a considerable capacity for insulin production persists. Conversely, in patients in whom the capacity for insulin production is limited or approaches zero, as in those with onset of diabetes in childhood, the drugs are not effective (30).

More specific and basic data indicating that the sulfonylurea compounds cause an increased output of insulin from the pancreas are to be found in a variety of studies. Pfeiffer *et al.* (31), extending their earlier studies on calves (32), placed cannulas in the femoral and portal veins of normal rats. Blood samples for the determination of sugar and of serum insulinlike activity were obtained before and 30 min. after the injection of a sulfonylurea compound (100 mg. of carbutamide or 10 mg. of metahexamide per kg.) into one of the tail veins. They found that, following the giving of the drug, there was a slight increase in the insulinlike activity of blood from the femoral vein and a very considerable rise in the portal vein. More recently, Stuhlfauth *et al.* (33) perfused the isolated pancreas of the dog with carbutamide and appropriate control solutions. They measured by paper chromatography the amount of insulin in the perfusate as it came from the vein leading away from the organ. They reported a definite output of insulin when carbutamide was perfused in a glucose-free solution. These findings are in general agreement with those of Goetz & Egdahl (34) who used tolbutamide and measured insulinlike activity by the mouse assay method. They are consistent also with the results of studies indicating that following the administration of sulfonylurea compounds there is a decrease in beta cell granulation [Volk & Lazarus (35)] and a decrease in the insulin content of the pancreas [Root (36)]. Furthermore, Colwell *et al.* (37) found that following the infusion of sulfonylureas into the pancreatic artery of dogs there was a definite reduction in the blood glucose,

ther clinical trial in the United States because of an incidence of toxic manifestations [Kirtley (15)] high enough to preclude further general usage. In June 1959, after a clinical trial of several months, metahexamide was likewise retired because of toxic effects, particularly on the liver (16, 17).

Sulfonylurea Compounds

Comparative pharmacology and metabolism.—The absorption of orally administered chlorpropamide and metahexamide is rapid and practically complete in 2 to 3 hr. Carbutamide aod tolbutamide are absorbed more slowly. However, the absorption of each of these four drugs is eventually almost complete [West & Johnson (18)]. With each, the concentration of the drug in the blood serum usually reacbes its peak level in 2 to 4 hr. after ingestion. Stowers, Constable & Hunter (19) stated that tbe volume of distribution of tolbutamide and chlorpropamide approximates the volume of the extracellular fluid of the body whereas carbutamide is distributed in about twice as large a volume. This was questioned by West & Johnson (18) who, having found that the serum levels of these three drugs were essentially the same at 40 min. after a rapid intravenous injection, concluded that the volumes of distribution of the drugs were quite similar. Metahexamide was included in the studies of West & Johnson; their data suggested tbat the volume of distribution of this compound might be ooly slightly lower than that of the other three.

Within the body, tolbutamide is readily metabolized to a carboxylic acid derivative that is physiologically inactive and excreted as such. Fajans (20) found tbat when doses of the order of 6 gm. per day are administered to human subjects, a maximum of about 75 per cent of the administered drug may be recovered from the urine as this oxidation product. On the other hand, it would appear that chlorpropamide undergoes no such chemical change but is slowly excreted io the urine unchanged. Whereas tolbutamide is carboxylated, carbutamide is acetylated, each in position 3 on the benzene ring [Achelis & Hardebeck (21)]. The metabolism of the sulfonylurea compounds varies somewhat from species to species.

The hypoglycemic effect of the sulfonylureas is, within certain limits, related to the level of the active form of the drug io the blood plasma. For tolbutamide, the effective level ranges from about 8 to 20 mg. per 100 ml. Concentrations above 20 mg. which may follow the giving of single doses larger than 2.0 to 3.0 gm. to human subjects, do not increase and may even decrease the hypoglycemic response [Baird & Duncan (22)]. If tolbutamide is giveo daily in dosage of 1.0 gm. and then discootinued, serum levels are very low after 24 hr. and undetectable after 48 hr. [Johnsoo *et al.* (23)]. Stowers *et al.* (19) state that the half life of tolbutamide, i.e., the time required for aoy given serum level to fall by 50 per cent, is 4 to 8 hr., that of chlorpropamide about 35 hr., and that of carbotamide 30 to 60 hr. with average values of 3.5, 34.5, and 40 hr. respectively. Hamwi *et al.* (24) reported that when siogle oral

Clinical use. In the early experience with the sulfonylurea agents, it was found that, in general, these drugs are effective in certain middle-aged and elderly persons with diabetes requiring no more than 40 or, more strictly, no more than 20, units of insulin daily for control [Mehnert, Camerini-Davalos & Marble (43)]. Early trials indicated that the compounds were not effective in most persons with onset of diabetes in childhood and adolescence, in other patients with unstable diabetes, in keto-acidosis and coma, during infections especially with fever, and during and following major surgical operations. Although many papers have been published during the last few years giving results in patients, very little of real importance has been added to these basic facts. It is true that some have reported effectiveness even during the conditions of stress just mentioned, particularly if combined with injections of insulin but, in such situations, the findings are often difficult to evaluate.

As in earlier reports, clinicians have stated that among selected patients with maturity-onset type of diabetes, the diabetic condition can be satisfactorily controlled in 50 to 80 per cent by means of appropriate doses of a sulfonylurea compound (44, 45). Lennon, Engbring & Engstrom (46) noted good to excellent control in 76 per cent of 104 selected patients treated with tolbutamide for 2 to 32 months. Some have reported (47) that a somewhat higher success rate was achieved with chlorpropamide than with tolbutamide. It is difficult, indeed, to compare the reported findings and impressions of one clinician with those of another because of the many factors which influence the outcome. Among such factors are the selection of patients, place of the clinical trial, i.e., whether in an outpatient department or under more carefully controlled conditions in a hospital, type of diet, frequency and interpretation of results of determinations of urine sugar and blood sugar, adequacy of data, and standards of control. Marble (48) has suggested the following standards: good control: majority of blood glucose values (Somogyi-Nelson method) fasting or at 3 or more hr. after food, must be 110 mg. per 100 ml. or below; fair control: majority of blood glucose values under the same conditions, 130 mg. per 100 ml.; poor control: all others. Somewhat similar standards were proposed by Skinner, Hayes & Hill (49) who designated degrees of control as follows: excellent: fasting blood sugar (Folin-Wu method), 120 mg. per 100 ml. or less; good: 120 to 140 mg; fair: 140 to 160 mg; and poor: more than 160 mg. per 100 ml. Pollen *et al.* (50) considered control good when the blood glucose was below 130 mg. per 100 ml. fasting, or below 150 mg. 2 hr. after food and when urine specimens obtained before meals showed only a trace of glucose or none at all. Control was considered fair when the blood glucose level was below 150 mg. per 100 ml. fasting, or below 180 mg. 2 hr. postprandially and when urine specimens before meals showed in general less than 0.5 per cent glucose. Other degrees of control were considered poor.

However, in many other reports, no definite blood glucose standards are stated. This is unfortunate because in the middle-aged and elderly persons under consideration, the renal threshold for glucose is often somewhat above the average normal so that tests for sugar in the urine may give an impression

whereas no such effect was obtained when similar small amounts were injected into peripheral vessels. They concluded that the sulfonylureas act probably by accelerating release of insulin from beta cells.

The statement that the sulfonylureas appear to stimulate the production or release of insulin is admittedly indefinite. Is production increased or is release facilitated, or do both occur? By what means are these accomplished? As Duncan & Baird (14) point out, answers to these questions are difficult because the basic mechanisms of insulin formation and discharge are as yet poorly understood.

Despite the considerable evidence to indicate stimulation of the production or release of insulin from the pancreas, studies designed to indicate increased peripheral glucose utilization in response to such insulin have, in general, yielded negative results. Although reports by various workers, published chiefly three or four years ago, are conflicting in some areas, for the most part studies including those of the arteriovenous difference, nitrogen balance, respiratory quotient, blood pyruvate, lactate, and phosphate, have failed to yield results that would indicate action of insulin in the periphery. To explain this discrepancy, the concept has arisen that the major action of the insulin released by sulfonylurea stimulation is in the liver. This has seemed reasonable in view of the fact that the pancreatoduodenal vein delivers blood into the portal vein, thence to the liver rather than directly into the peripheral circulation. This thought continues by suggesting that within the liver, insulin in physiological amounts acts by inhibiting the release of glucose into the blood. This problem was studied by Frawley *et al.* (38) who injected small amounts of insulin into the portal vein of human subjects undergoing abdominal surgery. It was found that the intraportal injection of insulin was followed by only a slight increase in the disappearance rate of D-xylose which had been infused in contrast to the marked effect produced by comparable doses injected into a peripheral vein. Madison *et al.* (39) concluded that insulin reaching the liver in physiological amounts from the pancreas by way of the portal blood inhibits hepatic glucose output. These workers, giving insulin to dogs with complete end-to-side portacaval shunts by slow intravenous infusion in a manner which minimized or prevented hypoglycemia and its attendant counter-regulatory response, found a prompt decline in hepatic glucose output.

That the presence of the liver is not essential for the hypoglycemic action of the sulfonylurea compounds has been shown in studies in which tolbutamide has been given to hepatectomized rats (40), dogs (41), and rabbits (42). However, available data suggest that in the intact subject, the lowering of the blood sugar following the administration of the sulfonylureas is caused chiefly by suppression of glucose output by the liver. This might be brought about, as discussed above, by the action of insulin released in response to sulfonylurea compounds or it might conceivably arise in part from the direct effect of the drugs themselves on the liver through a mechanism not yet elucidated.

incidence of side and toxic effects. In the order of their toxicity they may be listed as follows; metahexamide, carbutamide, chlorpropamide, and tolbutamide. As indicated earlier, carbutamide (58) and metahexamide were withdrawn from further clinical trial in the United States because of an incidence of toxic effects higher than the value of the drugs warranted. Carbutamide is still used in certain other countries and the relative safety of its use is defended (59).

Although there is a definite but small incidence of toxic effects from chlorpropamide, there have been very few reports of toxicity or of important side effects from tolbutamide during the five years in which this preparation has been used. Jaundice possibly related to tolbutamide has been described only rarely (43, 60), and then under unusual conditions with a past history of liver dysfunction. The case reported by Baird & Hull (60) was of the cholestatic type often seen in drug allergy. In this connection, it should be pointed out that studies of toxicity in animals cannot be applied directly in man. Thus, tolbutamide has been shown to possess well-marked hepato-toxicity in dogs, whereas in man as stated, there is little or no evidence of such. Sirek *et al.* (61) gave tolbutamide orally to 12 depancreatized dogs for prolonged periods of time. All of the animals developed impairment in liver function; 2 died and 6 had to be sacrificed because of jaundice and rapid deterioration. Apparently, tolbutamide is metabolized differently in dogs than in man [Mohnike & Wittenhagen (62)].

O'Donovan (44) summarized the side effects reported by cooperating physicians in clinical trials in 9168 patients, 57 per cent of whom had been maintained on tolbutamide for 6 to 28 months. Untoward responses were reported in 292 patients, 3.2 per cent of the total. Withdrawal of tolbutamide therapy was considered necessary in only 140 instances or 1.53 per cent. In these 140 patients the side effects were classified by O'Donovan as follows: hematologic, 11; dermatologic, 48; gastrointestinal, 63; other, 18. During the 28 months of observation a total of 136 patients died, but only in one instance could death be related definitely to the use of tolbutamide. This fatality was attributed to belatedly recognized hypoglycemia in an undernourished 86-year-old patient (63). No histologic evidence of islet cell damage attributable to tolbutamide administration could be demonstrated in those cases in which autopsy was performed. The incidence of side effects reported by O'Donovan agrees in general with that of Schöffling *et al.* (64) who reported from Frankfurt, Germany, their experience with 758 patients treated over a 20-month period with tolbutamide. These authors saw nothing to indicate that tolbutamide is likely to have any allergic or toxic action on the blood cells or bone marrow. Among the 758 patients, skin manifestations occurred as follows: urticaria, 7; dermatitis, 2; and toxic rashes, 3 patients. There were 7 instances of abdominal pain, 10 of pronounced constipation, 4 of diarrhea, and 1 prolonged episode of nausea. Schöffling *et al.* observed no instance of severe generalized allergic manifestations. The overall incidence of side effects necessitating withdrawal of tolbutamide was 1.6 per cent.

of better control than is actually present. Ricketts (51) has outlined the ideal procedure and requirements for the clinical testing of oral hypoglycemic agents.

Of general interest has been the matter of so-called "secondary failures" to the sulfonylurea compounds. This has been reported upon particularly with the preparation longest in use, tolbutamide. DeLawter *et al.* (52) have given their results with 200 patients who received tolbutamide for periods up to three years. The average maintenance dose was 1 gm. per day; this was increased to 3 gm. per day before the drug was considered ineffective. Following the first year of use, the secondary rate of failure was 29.5 per cent and the monthly failure rate averaged about 3 per cent of the patients treated each month. A satisfactory response to treatment with chlorpropamide or metahexamide was obtained in 20 per cent of those patients showing a secondary failure of response to tolbutamide. Herman & Jackson (53) used chlorpropamide in 43 patients in whom tolbutamide had not been effective. The response to chlorpropamide was considered excellent in 20 cases and "partial" in 14 others. The percentage of secondary failures as reported in the literature varies widely presumably because of differing programs of clinical trial. Various factors may contribute to secondary failures, among which are the unwise initial selection of patients and the failure of the patient to follow the prescribed diet. DeLawter *et al.* (52) found that patients who, during the initial regulation, achieved no better than poor or fair control were the ones most likely to develop secondary failures and this experience is in keeping with that reported by others (43).

The factor of adherence to diet is all-important. Except in patients with the lowest insulin requirements, the amount of carbohydrate and of total calories in the diet must be kept at appropriately restricted levels if sulfonylurea compounds are to have an adequate opportunity to be effective. Late failures arising from initial poor selection of patients or from non-adherence to diet are, strictly speaking, not drug failures. However, in addition to these types of secondary failures, there are those which occur without demonstrable cause. These possibly amount to 10 to 15 per cent of those patients initially well-controlled on the drug although accurate data on this point are lacking. The cause is obscure and in some patients may be an unexplained change in the basic character of the diabetic state. There has been no evidence brought forth to suggest that long-continued use of the sulfonylurea agents damages the islet cells of the pancreas (44) or impairs further the ability of the diabetic patient to utilize carbohydrate (54).

Use of sulfonylurea compounds in non-diabetic conditions.—Of interest are published accounts of the beneficial action of tolbutamide in acne vulgaris (55), Parkinson's disease (56), and multiple sclerosis (57). These reports await confirmation and clarification as to their mechanism of action.

Untoward and toxic effects.—Abundant clinical experience has demonstrated that the four sulfonylurea compounds, carbutamide, tolbutamide, chlorpropamide, and metahexamide have, in human patients, a varying

Place of sulfonylurea compounds in treatment.—Enthusiasm for hypoglycemic agents varies widely. Some clinicians make extensive use of these compounds. On the other hand, Duncan (71) considers that only approximately 10 per cent of all diabetic patients are "ideal" candidates for sulfonylurea therapy. Such patients are not overweight, have an adult-acquired, stable type of diabetes, and are satisfactorily controlled in 75 per cent of instances with either chlorpropamide or tolbutamide. Duncan estimates that 80 per cent of all diabetics are overweight and recommends reduction of the total caloric intake sufficient to reduce the overweight condition. He believes that dietary restriction alone is effective in controlling the diabetes, barring acute complications, in a majority of such patients and advises against supplementation of the undernutrition program with a sulfonylurea compound.

The Biguanides

The last few years have seen the introduction of oral hypoglycemic agents that are totally unrelated to the sulfonylureas. These compounds, the biguanides, of which the most widely used is phenformin (phenethylbiguanide; DBI), are among the guanidine derivatives. However, as will be brought out later in this discussion, the biguanides are non-toxic in human subjects in those doses that can be tolerated without digestive upsets. This is in sharp contrast to the toxic qualities of other hypoglycemic guanidine derivatives, including (*a*) the substituted monoguanidines such as methylguanide and (*b*) the alkylated biguanidines, Synthalin A and B, which were studied rather extensively some 30 years ago and used for a few years in the treatment of mild diabetes until their toxic effect, particularly on the liver, became apparent (72, 73).

The story of phenformin is not an old one since the first account of its effect in animals was published in 1957 by Ungar, Freedman & Shapiro (74). In the same year appeared a report of early trials in human diabetic patients begun in June, 1956, by Pomeranze, Fuiji & Mouratoff (75).

Pharmacology and toxicology.—Phenformin, administered parenterally or orally, causes a lowering of the blood sugar in guinea pigs, rats, rabbits, cats, and rhesus monkeys. In dogs, the hypoglycemic effect is inconstant at the dosage level made necessary by toxic effects which follow larger amounts (76). Phenformin is reported to be effective also in rats, rabbits, and monkeys made diabetic by means of alloxan (74); presumably, such animals retain some insulin-producing capacity.

In the majority of patients with diabetes, phenformin in dosage of 50 to 200 mg. by mouth causes some lowering of the blood sugar. Strange, and as yet unexplained, is the fact that in non-diabetic human beings phenformin has no hypoglycemic effect even when doses as large as 400 mg. a day are given (77, 78, 79).

A variety of biguanide derivatives have been studied for their ability to lower the blood sugar. Osterloh (80) investigated the hypoglycemic effect following the oral administration of a series of such compounds to normal

Mehnert *et al.* (43) found that among 594 patients maintained on tolbutamide for 1 to 20 months, the drug had been discontinued in 1.1 per cent because of untoward effects.

An untoward effect of some interest is that of unusual flushing of the skin, especially of the face and neck, following the taking of alcoholic beverages by a person receiving sulfonylurea compounds. This response is similar to that experienced by persons receiving disulfiram (Antabuse). It occurs to a greater extent with chlorpropamide than with tolbutamide (65). Dolger (66) states that the giving of an antihistaminic drug orally to susceptible individuals one hour prior to the takiog of alcohol will usually protect them from this side effect.

In the early experience with chlorpropamide, the incidence of side effects was higher than at the present time apparently because of the larger doses given at a time when clinical trials were in the exploratory stage. With daily doses of chlorpropamide of 1 gm. or more, some patients experienced lethargy, muscular weakness, ataxia, and dizziness. Other symptoms included anorexia, nausea, vomiting, abdominal discomfort, chest pain, skin rasbes, and leucopenia. A further hazard with larger doses was found to be prolonged hypoglycemia attributed to the slow metabolism of chlorpropamide within tbe body and the building up of an overly higb blood level of the drug. Following discussions at the confereoce on chlorpropamide and diabetes mellitus held in New York City on September 25–27, 1958 (9), considerably lower doses became the rule, and at the present time most clinicians do not exceed a daily dose of 500 mg. and find that smaller amounts often suffice. Even so, occasional instances of prolonged hypoglycemia have been reported in sensitive individuals. Karlin (67) described a case of fatal agranulocytosis. Another important complication of chlorpropamide therapy, encountered even with smaller daily doses, is tbat of hypersensitivity to the drug manifested usually by dermatitis or hepatic dysfunction, or both. In those patients who develope jaundice, the sequence of events has ofteo followed the same pattern. Most patients who exhibit drug idiosyncrasy to chlorpropamide will do so within two to five weeks after beginning treatment. Early symptoms and signs include a rise in tbe serum alkaline phosphatase, eosinophilia, skio rash, aod "drug fever." If recognized early eoough and tbe drug discontinued, overt jaundice may at times be avoided. The jauodice is usually of a benign, reversible type; liver biopsy has shown intracanalicular bile stasis (68). The patient reported by Rothfeld *et al.* (69) developed multiple toxic effects including jaundice of cholestatic type, erythema multiforme succeeded by exfoliative dermatitis, and olcerative proctocolitis with bloody diarrhea.

Among 382 patients treated with chlorpropamide, Blöch & Lenhardt (70) noted the following side effects: gastrointestinal, 9.7 per cent; dermatologic, 2.6 per cent; and alcohol intolerance (flushing of the face and oeck), 12 per cent. Despite periods of chlorpropamide usage up to 18 months, no instance of toxic effect on the liver, kidoey, or nervous system was recognized.

periods of time up to four years or more, nn permanent tissue toxicity, no hematopoietic effects, and no alteratinns in liver or kidney function have been reported.

Ketonuria may, at times, develop in patients receiving phenformin. This is thought to reflect inadequate carbohydrate utilization and can be overcome by increasing the amount of carbohydrate in the diet and perhaps the dose of insulin if such is being used concurrently (88).

Mode and site of action.—The blood sugar-lowering effect of phenformin and its analogues is easy to demonstrate in normal and diabetic animals and in certain diabetic patients. However, tn elucidate the mechanism and place of action of these compounds has proved to be a far more difficult matter. Williams *et al.* (89) have shown that in *in vitro* studies, phenformin causes an increased glucose uptake by the rat hemidiaphragm but, instead of an increase, there is a decrease in glycogen and an increased lactic acid production. According to Clarke & Forbath (90), the increase in glucose uptake is not accompanied by an increase in pentose space, insulin space, or water content of the intact isolated diaphragm. Steiner & Williams (91) interpreted their findings to indicate that the increased glucose uptake of the diaphragm might result from inhibition of oxidative activity rather than from a direct stimulation of anaerobic glycolysis. These workers found that the oxidation of several tricarboxylic acid cycle intermediates by mitochondria *in vitro* was inhibited in the presence of phenformin. They concluded that these compounds may produce hypoglycemia through a primary inhibitory effect on certain respiratory enzymes with cytochrome oxidase possibly a principal site of inhibition. However, Wick, Larson & Serit (92) thought that cytochrome oxidase is not inhibited but that the site of interference is at some point prior to cytochrome-*c* in the succinic oxidase system. Kruger *et al.* (93) prepared rat kidney mitochondria from isotonic sucrose homogenates and studied the effect of phenformin and dinitrophenol on the respiration of the mitochondria, using fumarate as a substrate. They found that phenformin inhibited oxygen uptake and that dinitrophenol released this inhibition. They consider their results to be consistent with the mechanism proposed by Hollunger (94) for the action of guanidine and its derivatives. Kruger *et al.* postulate that the effects of phenformin *in vitro* can best be explained as a "primary interference with the transfer of respiratory chain high-energy bonds to adenosinediphosphate, secondarily blocking electron transport which is reflected in diminution of oxygen uptake" with the creation of a partially anaerobic state. This diminishes the Pasteur effect which normally limits glycolysis in respiring cells.

When phenformin is added to liver preparations *in vitro*, a reduction in glycogen content and increased production of lactic acid are observed [Williams *et al.* (83)]. Nielson *et al.* (87) showed that phenformin produces a marked decrease in the output of glucose from the liver, and work from the same laboratory (89) indicated that this is attributable neither to inhibition of glucose-6-phosphatase nor to increased storage of liver glycogen. As a

mice, rats, and guinea pigs. He found that 1-butyl-biguanide in comparable dosage had a considerably greater blood sugar-lowering effect than phenethylbiguanide. The compound, 1-butyl biguanide, has been subjected to extensive clinical trials in Germany (81). In the United States, the hypoglycemic action of amyl-(DBB), isoamyl-(DBTU), and methylbenzyl-(DBC) as well as butyl-(DBV) biguanide has been used with diabetic patients on an investigational basis but found not to have any important advantage over phenformin (82, 83).

Penhos & Blaquier (84) in Houssay's laboratory studied the toxicity of phenformin and determined the lethal dose in dogs, rats, guinea pigs, cats, and rabbits following both oral and (in some animals) subcutaneous administration. They state that when large doses are given, toxicity arises mainly from hypotension and from action on the nervous system, with an additional factor of hypoglycemia in rats (85). Hypophysectomized dogs and adrenalectomized dogs and rats were more sensitive to phenformin than were normal animals. Adrenalin, glucose, and hydrocortisone had a protective effect on the mortality of rats, but not on that of dogs in the doses used. They found no hypoglycemic effect in depancreatized dogs.

Ungar *et al.* (74) found early that evaluation of toxicity of phenformin is made difficult by the fact that animals die in hypoglycemia before other toxic effects can be observed. Postmortem examination of tissues from guinea pigs and a monkey dying in hypoglycemia after a large dose of the drug showed no damage in kidney, adrenals, liver, spleen, intestine, heart, pancreas, skeletal muscle, lungs, testes, or lymph nodes. Likewise, Volk & Lazarus (86) reported that phenformin does not cause histologic or histochemical alterations in pancreas, liver, or skeletal muscle and, furthermore, that no changes were observed in the kidney after the giving of moderate doses of the drug. In two rabbits given large amounts of phenformin (100 mg. per kg. twice daily for 6 days), hyaline droplet formation and vacuolization of the distal convoluted tubules as well as a reduction in succinic dehydrogenase activity in the kidneys were found. There is evidence that animals can tolerate, over long periods of time, doses of phenformin which, compared to those used in human diabetic patients, are very large. Phenformin given for periods up to six months causes no demonstrable biochemical or histological damage in rats, guinea pigs, rabbits, monkeys, or dogs. Nielson *et al.* (87) found the growth of young guinea pigs to be unimpaired by the administration of phenformin.

In human subjects, as already noted, there is a high incidence of gastrointestinal symptoms following phenformin if doses above a certain level are exceeded. Symptoms consist of anorexia, nausea, vomiting and, at times, abdominal distress and diarrhea. The dose that is tolerated varies from person to person but the unpleasant side effects occur frequently if the total dosage given to an adult exceeds 200 mg. daily with a still higher incidence of untoward effects if the dose is 300 mg. or greater.

Although phenformin has now been used with thousands of patients for

chosen, was achieved in 88 per cent with or without concomitant treatment with insulin. A similar optimistic report was that of Pomeranze *et al.* (100) who used phenformin in 206 patients for two years. Confirming the earlier impression of this group of workers (101), phenformin was considered to be an effective hypoglycemic agent in all types of diabetic patients. Among the 206 persons mentioned, 128 were under treatment which the authors considered successful at the time of the report. Of the 128 patients, six had been followed for more than two years, 18 for more than one year and the remainder for 1 to 12 months. A total of 110 patients among the group of 128 were treated with phenformin alone; 68 of these were over the age of 45 years.

Although it may well not be true of physicians generally, a survey of the literature suggests that the majority of those who devote a large share of their attention to the treatment of diabetes and who use phenformin at all, employ it chiefly in the following classes of patients (102): (*i*) Those with unstable diabetes, usually persons with onset of diabetes below the age of 30 years. In such patients, insulin is continued although often in lower dosage. (*ii*) Those in whom a "secondary failure" has developed with a sulfonylurea compound. (*iii*) Those in whom sulfonylureas are partially effective but in whom the giving of phenformin along with a sulfonylurea compound results in satisfactory control of hyperglycemia and glycosuria.

Krall *et al.* have reported their results in a series of papers (103 to 108). In one of the latest of these publications (107), Krall summarized the experience with more than 350 patients who had received a biguanide compound in the 28-month period from December 1956 to April 1959. Among the 244 patients of the series on whom adequate data were available, there were many in whom the diabetic condition was unstable and difficult to control. A significant blood sugar-lowering effect was observed in 88 per cent of the group. However, at the time of the report only 107 patients were still receiving the biguanide preparation with duration of such treatment ranging from 3 to 28 months. Of the 107, 58 were under treatment with dietary restriction and biguanides alone while 49 others were receiving insulin as well as the oral agent. Krall regards phenformin as particularly useful in smoothing the course of patients with unstable diabetes. Such an effect is admittedly difficult to document with concrete data.

As summarized by Beaser (109), five clinicians speaking at a symposium in Houston, Texas, early in 1959 reported the frequent finding of a reduction in insulin needs when phenformin was given concomitantly. The same workers noted a stabilizing effect of phenformin on "brittle" diabetes among a group of diabetic patients totaling 145, and specifically studied for this purpose. These satisfactory features of phenformin treatment were noted particularly by those dealing with the growth-onset (juvenile) type of diabetes.

The combination of treatment with a sulfonylurea agent and phenformin has been reported upon particularly by Beaser (110) and by Mehnert (111). Their findings are in general agreement with those of others, to the effect that

matter of fact, in fasted guioea pigs, phenformin causes a marked depletion of liver glycogen. Williams, Tanner & Odell (95) concluded that phenformin probably produces hypoglycemia mainly in two ways: (*a*) by promoting anaerobic glycolysis, witb increased glucose utilization, and (*b*) by decreasing gluconeogenesis.

Despite the fairly consistent results of *in ritro* studies which seem to show that phenformin may inhibit electron transport and enzymatic oxidation at the succinic debydrogenase or cytochrome oxidase level, or both, in muscle, adipose tissue, and liver, Ungar, Psycboyos & Hall (96) have expressed great doubt as to tbe validity of the idea that the hypoglycemic action of phenformio arises from inhibition of tissue oxidation. They base this opinion on the findiogs that certain biguanides which have a hypoglycemic effect *in rivo* have little or no inhibitory effect on tissue oxidation *in ritro*, and vice versa. Beckmann (97) agrees witb Ungar in this regard and, in additioo, points out the fact that the amounts of biguanide used in the *in ritro* studies have been uophysiologically higb. He regards the mode of action of the biguanides as still not clear but, in fact, "völlig im dunkeln."

Clinical use.—There is geoeral agreement that phenformin exerts some blood sugar-lowering effect io a high percentage of persons of varying ages and durations of diabetes (12, 13, 98). In certain middle-aged and elderly persons with "mild" diabetes, presumably those with considerable endogenous insulin production, phenformin may be used alone. In other diabetic patieots, particularly children and older persons with the juvenile, unstable type of diabetes, pbenformin may be effective if insulio in dosage suited to the iodividual, is given. In such patients, tbe giving of phenformin may decrease the iosulio requiremeot by 25 to 50 per cent. As previously noted, a high incidence of side effects consistiog of a metallic taste, anorexia, oausea, vomiting, aod diarrhea may be expected if daily doses are given which exceed (in adults) 150 to 200 mg. per day and almost certainly if the dose exceeds 300 mg. per day. Smaller doses must be used with children, and in all patients the total daily amouot must be given in three or four doses daily. Uoder clinical trial at present are "time disintegratioo" capsules containing either 50 or 100 mg. of the drug. This simplifies treatment since, io respoosive patieots, only one capsule at breakfast and ooe at supper giviog a total of either 100 or 200 mg. of the drug daily, are necessary.

At the present writing, there is a wide divergeoce of opinion among clinicians as to the place and value of pheoformio in the treatment of diabetes. At one extreme are those who look with disfavor upon any use of the biguanides and, oo the other band, there are those who, like Barclay (99), helieve that "phenformin is a safe and effective orally given hypoglycemic drug which is sufficieotly wide in its range of activity to he considered the oral therapy of choice io the management of diahetes." Barclay reported his results witb 104 patieots aged 4 to 83 years who were studied for seven months. Most of these patients had had difficulty in controlliog diahetes prior to taking pheoformin. Fair to excellent control, accordiog to tbe standards

26. Loubatières, A., *Ann. N. Y. Acad. Sci.*, 71, 4–11 (1957)
27. Houssay, B. A., and Migliorini, R. H., *Rev. soc. arg. biol.*, 32, 94–99 (1956)
28. Mukherjee, S. K., De, U. N., and Mukerji, B., *Indian J. Med. Research*, 46, 57–62 (1958)
29. Ricketts, H. T., Wildberger, H. L., and Schmid, H., *Ann. N. Y. Acad. Sci.*, 71, 170–76 (1957)
30. Camerini-Davalos, R., Marble, A., White, P., Belmonte, M., and Sargeant, L., *New Engl. J. Med.*, 256, 817 (1957)
31. Pfeiffer, E. F., Pfeiffer, M., Ditschuneit, H., and Ahn, C-S., *Ann. N. Y. Acad. Sci.*, 82, 479–95 (1959)
32. Pfeiffer, E. F., Steigerwald, H., Sandritter, W., Bänder, A., Becker, U., and Retiene, K., *Deut. med. Wochschr.*, 82, 1568–74 (1957)
33. Stuhlfauth, K., Mehnert, H., Schäffer, G., and Kaliampetsos, G., *Klin. Wochschr.*, 38, 825–26 (1960)
34. Goetz, F. C., and Egdahl, R. H., *Federation Proc.*, 17, 55 (1958)
35. Volk, B. W., and Lazarus, S. S., *Diabetes*, 6, 125–28 (1957)
36. Root, M. A., *Diabetes*, 6, 12–16 (1957)
37. Colwell, A. R., Jr., Colwell, J. A., and Colwell, A. R., Sr., *Metabolism*, 5, 727–32 (1956)
38. Frawley, T. F., Shelley, T. F., Runyan, J. W., Jr., Margulies, E. J., and Cincotti, J. J., *Ann. N. Y. Acad. Sci.*, 82, 460–78 (1959)
39. Madison, L. L., Combes, B., Adams, R., and Strickland, W., *J. Clin. Invest.*, 39, 507–22 (1960)
40. Dulin, W. E., and Johnston, R. L., *Ann. N. Y. Acad. Sci.*, 71, 177–91 (1957)
41. Sobel, G. W., Rodriguez-Inigo, J., and Levine, R., *Metabolism*, 7, 222–26 (1958)
42. Richter, H., *Naturwissenschaften*, 45, 165 (1958)
43. Mehnert, H., Camerini-Davalos, R., and Marble, A., *J. Am. Med. Assoc.*, 167, 818–27 (1958)
44. O'Donovan, C. J., *Current Therap. Research*, 1, 69–87 (1959)
45. Shlevin, E. L., Zarowitz, H., Weisenfeld, S., and Goldner, M. G., *Metabolism*, 9, 570–79 (1960)
46. Lennon, E. J., Engbring, N. H., and Engstrom, W. W., *Wisconsin Med. J.*, 59, 191–96 (1960)
47. Fineberg, S. K., *J. Am. Geriat. Soc.*, 8, 441–48 (1960)
48. Marble, A., *Med. Clin. North Am.*, 42, 1163–77 (1958)
49. Skinner, N. S., Jr., Hayes, R. L., and Hill, S. R., Jr., *Ann. N. Y. Acad. Sci.*, 74, 830–44 (1959)
50. Pollen, R. H., Barnes, R. H., Tanner, D. C., Stimson, W. H., and Williams, R. H., *Diabetes*, 9, 25–30 (1960)
51. Ricketts, H. T., *Diabetes*, 8, 472–73 (1959)
52. DeLawter, De W. E., Moss, J. M., Tyroler, S., and Canary, J. J., *J. Am. Med. Assoc.*, 171, 1786–92 (1959)
53. Herman, J. B., and Jackson, W. P. U., *S. African Med. J.*, 34, 31–34 (1960)
54. Gorman, C. K., and Weaver, J. A., *Brit. Med. J.*, II, 1214–17 (1959)
55. Cohen, J. L., and Cohen, A. D., *Can. Med. Assoc. J.*, 80, 629–32 (1959)
56. Gates, E. W., and Hyman, I., *J. Am. Med. Assoc.*, 172, 1351–54 (1960)
57. Sawyer, G. T., *J. Am. Med. Assoc.*, 174, 470–73 (1960)
58. Marble, A., and Camerini-Davalos, R., *Ann. N. Y. Acad. Sci.*, 71, 239–48 (1957)
59. Stratmann, F. W., *Medizinische*, 21, 1014–17 (1959)
60. Baird, R. W., and Hull, J. G., *Ann. Internal Med.*, 53, 194–96 (1960)
61. Sirek, A., Sirek, O. V., Hanus, Y., Monkhouse, F. C., and Best, C. H., *Diabetes*, 8, 284–88 (1959)
62. Mohnike, G., and Wittenhagen, G., *Deut. med. Wochschr.*, 82, 1556–57 (1957)
63. McKendry, J. B. R., Kuwayti, K., and Sagle, L. A., *Can. Med. Assoc. J.*, 77, 429–38 (1957)
64. Schöffling, K., Pfeiffer, E. F., Steigerwald, H., Bachrach, I., and Becker, U., *Deut. med. Wochschr.*, 82, 1537–39 (1957)
65. Signorelli, S., *Ann. N. Y. Acad. Sci.*, 74, 900–3 (1959)
66. Dolger, H., *J. Am. Med. Assoc.*, 173, 1278 (1960)
67. Karlin, H., *New Engl. J. Med.*, 262, 1076–77 (1960)
68. Brown, G., Zoldis, J., and Spring, M., *J. Am. Med. Assoc.*, 170, 2085–88 (1959)
69. Rothfeld, E. L., Goldman, J., Goldberg, H. H., and Einhorn, S., *J. Am. Med. Assoc.*, 172, 104–6 (1960)
70. Blöch, J., and Lenhardt, A., *Wiener med. Wochschr.*, 110, 101–5 (1960)
71. Duncan, G. G., *Am. J. Med. Sci.*, 239, 397–402 (1960)

the addition of phenformin treatment to that with a sulfonylurea compound may result in satisfactory control of the urine and blood sugar in patients in whom either a secondary failure to the sulfonylurea agent has occurred or in whom only partial success has been obtained with this type of compound. Viewing the matter from another point of view, Mehnert believes that the giving of a sulfonylurea compound to a patient receiving biguanides may permit a lower dosage of the latter with lessening of gastrointestinal side effects.

As with the sulfonylurea compounds, one serious fault in many clinical trials has been the lack of carefully established standards in the rating of results of treatment. Furthermore, standards, when selected and reported in publications, have often been far from strict, and those chosen by different investigators have varied so widely that it is difficult to compare the results obtained by one worker with those of others (98).

Experience with the oral hypoglycemic agents during the past five years has been extraordinarily fruitful, not only from the standpoint of their use with patients but also because of the attention which has been directed anew to the fundamental problems of diabetes and the action of insulin. It is obvious that many questions remain unanswered and that more time is required to determine the proper place of the various compounds in the treatment of diabetes.

LITERATURE CITED

1. Franke, H., and Fuchs, J., *Deut. med. Wochschr.*, 80, 1449–52 (1955)
2. Bertram, F., Bendfeldt, E., and Otto, H., *Deut. med. Wochschr.*, 80, 1455–60 (1955)
3. Maske, H., *et al*, *Deut. med. Wochschr.*, 81, 823–46, 887–906 (1956)
4. Levine, R., Duncan, G. G., *et al.*, *Metabolism*, 5, 721–977 (1956)
5. Best, C. H., *et al.*, *Can. Med. Assoc. J.*, 74, 957–98 (1956)
6. Peck, F. B., Sr., *et al.*, *Diabetes*, 6, 1–94 (1957)
7. Levine, R., *et al.*, *Ann. N. Y. Acad. Sci.*, 71, 1–292 (1957)
8. Mohnike, G., *et al.*, *Deut. med. Wochschr.*, 82, 1513–92 (1957)
9. Goldner, M. G., *et al.*, *Ann. N. Y. Acad. Sci.*, 74, 407–1028 (1959)
10. Ashmore, J., *et al.*, *Metabolism*, 8, 469–686 (1959)
11. Forsham, P. H., *et al.*, *Ann. N. Y. Acad. Sci.*, 82, 191–644 (1959)
12. Symposium on Phenformin, *Diabetes*, 9, 163–227 (1960)
13. Bertram, F., and Michael, G., *Intern. Biguanid-Symposium* (Georg Thieme Verlag, Stuttgart, Germany, 167 pp., 1960)
14. Duncan, L. J. P., and Baird, J. D., *Pharmacol. Revs*, 12, 91–158 (1960)
15. Kirtley, W. R., *Diabetes*, 6, 72–73 (1957)
16. Moss, J. M., and DeLawter, D., *Ann. N. Y. Acad Sci.*, 82, 614–17 (1959)
17. Mach, B., Field, R. A., and Taft, E. B., *New Engl. J. Med.*, 261, 438–40 (1959)
18. West, K. M., and Johnson, P. C., *Metabolism*, 8, 596–605 (1959)
19. Stowers, J. M., Constable, L. W., and Hunter, R. B., *Ann. N. Y. Acad. Sci.*, 74, 689–95 (1959)
20. Fajans, S. S., *Ann. N. Y. Acad. Sci.*, 74, 471–72 (1959)
21. Achelis, J. D., and Hardebeck, K., *Deut. med. Wochschr.*, 80, 1452–55 (1955)
22. Baird, J. D., and Duncan, L. J. P., *Scot. Med. J.*, 2, 314–50 (1957)
23. Johnson, P. C., Hennes, A. R., Driscoll, T., and West, K. M., *Ann. N. Y. Acad Sci.*, 74, 459–72 (1959)
24. Hamwi, G. J., Skillman, T. G., Freedy, L. Y., and Alexander, L. A., *Metabolism*, 8, 631–43 (1959)
25. Loubatières, A., *Presse méd.*, 63, 1701–3, 1728–30 (1955)

EVALUATION OF HAZARDS OF RADIATION EXPOSURE IN MEDICAL PRACTICE[1]

By L. H. Hempelmann, M.D.

Division of Experimental Radiology, University of Rochester School of Medicine and Dentistry, Rochester, New York

Since the Atomic Age began, the medical profession has become increasingly aware of the potential dangers resulting from exposure of ever-larger numbers of people to ionizing radiation. Now that the entire world's population is exposed, to a greater or lesser degree, to the radiations from the fallout of hydrogen bomb testing, the hazards of radiation have become as familiar to the reading public as to the physician. It came as a distinct shock, however, when, in 1956, two national committees, one in Great Britain (1) and one in this country (2), disclosed the fact that the estimated average dose of radiation received by each individual in these countries from medical sources far exceeded that from any other man-made source. With the realization that x-rays used even diagnostically might be harmful, it became necessary to reassess the actual dangers involved and to reconsider how the risks of medical radiography and radiation therapy might be reduced without denying the admitted benefits of such procedures. The purpose of this paper is to attempt to assess these risks using, as far as possible, data obtained from studies of the effect of radiation on man.

Although it is sometimes said that radiation injuries are not well understood, it must be realized that the biological phenomena seen as a result of radiation injury are probably as well established as those of any other disease. A vast amount of research has been conducted on radiation-induced biological reactions, ranging from the submolecular through mammalian levels and including extensive studies in man. This has given us an immense amount of knowledge about the biological mechanism involved in radiation injury. What is not clearly understood, however, is the quantitative relationship between the dose of radiation and the degree of injury, particularly at low-radiation levels. The fact that valid data are difficult if not impossible to accumulate at near background radiation levels is what has led to the controversial statements that one reads in scientific articles as well as in lay writings. In an attempt to provide such badly needed biological information, scientists have extrapolated data obtained using high doses to low levels to predict quantitative reactions in man. It is in these areas where knowledge is limited and where conclusions and convictions are often intuitive, that one reads and hears contradictory and misleading statements about radiation hazards.

To illustrate the difficulty of assessing hazards to man of exposure to low

[1] The survey of the literature pertaining to this review was concluded in August, 1960.

72. Bertram, F., *Med. Klin.*, 24, 1229–32 (1928)
73. Mahler, R. F., *Brit. Med. Bull.*, 16, 250–54 (1960)
74. Ungar, G., Freedman, L., and Shapiro, S. L., *Proc. Soc. Exptl. Biol. Med.*, 95, 190–92 (1957)
75. Pomeranze, J., Fujii, H., and Mouratoff, J. T., *Proc. Soc. Exptl. Biol. Med.*, 95, 193–94 (1957)
76. Houssay, B. A., and Penhos, J. C., *Rev. soc. arg. biol.*, 34, 53–63 (1958)
77. Fajans, S. S., Moorhouse, J. A., Doorenbos, H., Louis, L. H., and Conn, J. W., *Diabetes*, 9, 194–201 (1960)
78. Madison, L. L., and Unger, R. H., *Diabetes*, 9, 202–6 (1960)
79. Otto, H., *Intern. Biguanid-Symposium*, 97–101 (Georg Thieme Verlag, Stuttgart, Germany, 1960)
80. Osterloh, G., *Intern. Biguanid-Symposium*, 8–11 (Georg Thieme Verlag, Stuttgart, Germany, 1960)
81. Relevant papers, *Intern. Biguanid-Symposium* (Georg Thieme Verlag, Stuttgart, Germany, 1960)
82. Krall, L. P., and Bradley, R. F., *Diabetes*, 7, 468–77 (1958)
83. Williams, R. H., Tanner, D. C., and Odell, W. D., *Diabetes*, 7, 87–92 (1958)
84. Penhos, J. C., and Blaquier, J. A., *Rev. soc. arg. biol.*, 34, 21–28 (1958)
85. Ashkar, E., Burrier, C. N., and Ramos, M. C. de P., *Rev. soc. arg. biol.*, 34, 11–20 (1958)
86. Volk, B. W., and Lazarus, S. S., *Diabetes*, 9, 174–77 (1960)
87. Nielson, R. L., Swanson, H. E., Tanner, D. C., Williams, R. H., and O'Connell, M., *Arch. Internal Med.*, 101, 211–15 (1958)
88. Goldner, M. G., Baldwin, R. S., Dobson, H. L., Krall, L. P., Lambert, T. H., Miller, E. C., Pomeranze, J., and Weller, C., *Diabetes*, 9, 220–21 (1960)
89. Williams, R. H., Tyberghein, J. M., Hyde, P. M., and Nielson, R. L., *Metabolism*, 6, 311–19 (1957)
90. Clarke, D. W., and Forbath, N., *Diabetes*, 9, 167–69 (1960)
91. Steiner, D. F., and Williams, R. H., *Biochim. et Biophys. Acta*, 30, 329–40 (1958)
92. Wick, A. N., Larson, E. R., and Serif, G. S., *J. Biol. Chem.*, 233, 296–98 (1958)
93. Kruger, F. A., Skillman, T. G., Hamwi, G. J., Grubbs, R. C., and Denforth, N., *Diabetes*, 9, 170–73 (1960)
94. Hollunger, G., *Acta Pharmacol. Toxicol.*, 11, Suppl. 1, 1–84 (1955)
95. Williams, R. H., Tanner, D. C., and Odell, W. D., *Diabetes*, 7, 87–91 (1958)
96. Ungar, G., Psychoyos, S., and Hall, H. A., *Metabolism*, 9, 36–51 (1960)
97. Beckmann, R., *Intern. Biguanid-Symposium*, 12–16 (Georg Thieme Verlag, Stuttgart, Germ., 1960)
98. Marble, A., *Diabetes*, 9, 225–27 (1960)
99. Barclay, P. L., *J. Am. Med. Assoc.*, 174, 474–80 (1960)
100. Pomeranze, J., Mouratoff, G. T., Gadek, R. J., and King, E. J., *J. Am. Med. Assoc.*, 171, 252–60 (1959)
101. Pomeranze, J., Sujiy, H., and Mouratoff, G. T., *Proc. Soc. Exptl. Biol. Med.*, 95, 193–94 (1957)
102. von Planta, von F., *Intern. Biguanid-Symposium*, 145–48 (Georg Thieme Verlag, Stuttgart, Germany, 1960)
103. Krall, L. P., and Camerini-Davalos, R., *Proc. Soc. Exptl. Biol. Med.*, 95, 345–47 (1957)
104. Krall, L. P., and Camerini-Davalos, R., *Arch. Internal Med.*, 102, 25–31 (1958)
105. Krall, L. P., White, P., and Bradley, R. F., *Diabetes*, 7, 468–77 (1958)
106. Krall, L. P., and Bradley, R. F., *Ann. Internal Med.*, 50, 586–613 (1959)
107. Krall, L. P., *Ann. N. Y. Acad. Sci.*, 82, 603–13 (1959)
108. Krall, L. P., Bradley, R. F., and White, P., *Intern. Biguanid-Symposium*, 86–93 (Georg Thieme Verlag, Stuttgart, Germany, 1960)
109. Beaser, S. B. *et al.*, *Diabetes*, 9, 222–24 (1960)
110. Beaser, S. B., *New Engl. J. Med.*, 259, 1207–10 (1958)
111. Mehnert, H., *Intern. Biguanid-Symposium*, 122–26 (Georg Thieme Verlag, Stuttgart, Germany, 1960)

radiology were particularly guilty of this, especially in the course of fluoroscopic examinations. For example, it has been estimated that many children in one American city must have received x-ray doses of the order of 100 roentgens to their entire bodies during the first year of life in the course of routine fluoroscopic examinations in Well-Baby Clinics (3). With increasing awareness of the potential dangers on the part of physicians, with the improved radiological techniques, and with the actions taken by Public Health Departments in a number of states, exposure of patients in general has been reduced considerably in the last five years. Thus, in Richmond, Washington, a town well aware of radiation hazards because of its connection with the Atomic Energy Plant at Hanford, the average gonadal dose to residents in the course of one year was calculated to be 1.3 r during the first 30 years of life (4) rather than the 4.6 r estimated to be the average gonadal dose throughout the country (5). With the current and projected improvement in x-ray equipment, increased sensitivity of films, and measures such as protection of gonads, there is every reason to believe that the contribution of medical radiography to the total exposure of our population will continue to decrease.

With this background in mind, an attempt will now be made to assess the actual danger to large population groups exposed repeatedly to radiation at low levels such as are used in medical radiography.

Genetic Effects

In 1927, Muller reported that exposure of adult male fruit flies to x-rays increased the spontaneous mutation rate (6). This mutagenic action of radiation has been confirmed repeatedly in many species of animals including mice. The following summary of these genetic effects of radiation in animals especially pertinent to the problem in man is taken largely from the United Nations Report (7) and from publications of W. L. Russell (8 to 11).

There appears to be no threshold dose below which radiation does not cause mutations. The mutagenic effect of radiation on spermatogonia and oocytes appears to be cumulative throughout the reproductive life of the exposed animal. Almost all detectable mutations produced by radiation are harmful to some degree. The dose of radiation that will double the spontaneous incidence of mutations varies with the species but tends to cluster around the range of 30 to 60 roentgen equivalents.

A given dose of radiation will induce 15 times as many point mutations in spermatogonia of mice as in the corresponding cells of fruit flies (8). Recently it has been shown that fewer mutations were produced in the spermatogonia of mice by a given dose of x-rays administered at a rate of 90 r per minute than by the same total dose given at a rate of 90 r per week (9). However, reducing the exposure rate to 10 r per week did not further diminish the frequency of mutation (10). Finally, it has been shown that certain types of radiation reduce the life-span of the offspring not yet conceived as well as that of the irradiated parent mice (11).

doses of radiation, it must be emphasized that the quantitative data relating dose to response must be obtained directly from studies in man himself. Although all phenomena seen in persons ill from radiation exposure have been observed in animals, direct extrapolation of such animal data to man is not valid because of the well-known species variation in susceptibility to radiation response. Even in animals, individual variability makes it necessary to study groups of animals to establish quantitatively the response to graded doses of radiation. In man, groups of irradiated individuals available for study are rare, particularly those having received known doses of radiation. In order to detect subtle changes caused by radiation, such as an increase in an uncommon disease, for example, leukemia, or life-shortening by a fraction of the total life span, it is necessary to study population groups numbering in the thousands or tens of thousands. Usually, in these groups with known exposure such as the Japanese in Hiroshima and Nagasaki, or radiologists, the individual doses are inexact. In groups of patients treated with x-rays or radium the exact doses are usually known, but here the response to exposure of a limited part of the body cannot be compared quantitatively or even qualitatively to exposure of the entire body.

Study of large population groups is subject to all the difficulties inherent in epidemiological surveys. The long period of time elapsing between exposure and the phenomenon under investigation adds immeasurably to the complexity of the study, especially since almost all members of the group must be located in order to demonstrate the presence or absence of all but the most obvious forms of radiation effects. Since one only proves an association between radiation exposure and the effect and does not establish a cause-and-effect relationship, one cannot differentiate clearly between the effect of the disease itself and that of the radiation treatment on the end-point being studied, in patients exposed to radiation for therapeutic purposes. Selection of a proper control group should facilitate such a decision but in practice adequate control groups are virtually unavailable and the groups selected are usually open to criticism. Finally, one can only observe the natural phenomena occurring in the life of these groups and cannot subject persons to procedures which might give evidence of subclinical or preclinical radiation damage. Particularly, this makes demonstration of genetic effects in man of any sort except the most obvious difficult if not impossible.

As has been mentioned, radiography for medical purposes contributes substantially to the cumulative radiation dose received by a population with advanced medical care. Until the publication of the reports of the two committees mentioned above, little thought was given to the potential risks of such medical exposures even though individual geneticists such as H. J. Muller had pleaded the case often and eloquently. Although the radiation doses received by individuals even for therapy were rarely sufficient to cause recognizable signs of injury, it is obvious that failure to recognize the possibility of untoward late effects resulted in far more exposure of patients than was absolutely necessary. Physicians with little or no formal training in

other traits such as cleft palate which concentrate in families but do not follow simple genetic theory. Environmental factors during embryonal development or the involvement of many genes modifying the developmental process in a complex manner probably play a role in this and other cases. Other genetic diseases such as cystic fibrosis of the pancreas, although following the laws of recessive gene transmission in families, occur too frequently to be explained by a balance between mutation and natural selection. A final large group of hereditary diseases including schizophrenia and manic depressive psychoses have been attributed to simple recessive mutant genes modified in some way to an unknown extent in their expression, and by a mechanism which is not clear.

In addition to these sharply defined traits controlled by specific genes, there are other human characteristics which depend upon genetic control of a different type. These characteristics, such as life-span, intelligence, birth weight, and stature, are determined by many genes each with an effect so small and so closely related to each other or to the environment that only the collective effect of the influence of the genes as a group can be studied by statistical methods. Although the effect of an increased mutation rate on traits controlled by specific genes can be calculated with some degree of certainty, the effect on the characteristics just described is not at all clear.

Perhaps the most important concept for the physician to understand in order to appreciate the genetic hazards of radiation is that concerned with the burden of mutant genes in the gene "pool" of the total population. Each mutant gene not leading to death of the individual in the first generation or to his failure to reproduce is passed on from parent to offspring until eventually, no matter how small its deleterious effect, it will lead to the premature death of a descendant or to his failure to produce the normal number of offspring.[3] These so-called "genetic deaths" must balance the number of new mutant genes being introduced into the population. This results in a state of equilibrium which we do not want to disturb by subjecting the population to the action of mutagenic agents such as man-made radiation or certain chemicals. It is implicit in the concept of the mutant gene "pool" that each unit of radiation absorbed in the germ tissues is equally effective (ignoring differences caused by dose rate) in adding to the genetic burden of the population whether one person receives a large dose (provided he is not sterilized) or whether many people receive smaller amounts which add up to the same total dose. Thus, the genetic burden to society of exposure of persons to a given dose of radiation will depend upon the fraction of the total population the irradiated group represents. With increasing prevalence of man-made radiation and now radioactive "fallout" from hydrogen bomb tests exposing essentially the entire world's population, the effect on the mutant gene pool is much greater even with small exposures per individual than when only a

[3] The number of recessive mutant genes per person has been estimated to be as high as 60 by Muller (16) and as low as 0.5 by Stevenson (15).

or less can cause malignant change or have other undesirable late effects. As a result of two studies of man, to be reviewed below, it has even been suggested that the leukemogenic action of radiation has no threshold. Further, if certain assumptions are made about the dose in these studies, it can be postulated that the dose of radiation to the entire body required to double the spontaneous incidence of leukemia is of the order of that required to double the mutation rate in germ cells. These hypotheses challenge the classical view that the somatic effect of radiation is a threshold reaction whereas the genetic effect is non-threshold.

Four somatic effects of radiation will now be considered. They include the effect on embryonic development, tumor induction, fertility, and shortening of life span. As before, studies in man will be emphasized and, where possible, the effects in the low dose range will be stressed.

Developmental abnormalities.—Animal experiments have shown that the embryo and fetus are exceedingly sensitive to the effect of radiation. Irradiation during the preimplantation period causes death of most of the litter; those animals which survive to birth are not grossly malformed (20). In contrast, irradiation during the postimplantation period produced developmental abnormalities, with those involving the skeleton and cerebral nervous system being especially prominent (20, 21). The former are caused by as little as 25 r of x-rays (20). The embryo is most radiosensitive during the period of major organogenesis. There is a critical period for irradiation during development which governs the type of abnormality produced. It should be pointed out that this effect of radiation is not unique as similar malformation is produced by radiomimetic drugs, nutritional imbalance, etc.

In the case of the human embryo, the period of major organogenesis lasts from approximately the second week to the sixth or seventh week (20). This would be expected to be the period of maximum radiosensitivity. There are numerous isolated clinical examples of anomalies in children exposed prenatally to radiation (20, 21). Six of eleven children of Japanese mothers at Hiroshima within 1200 m. of the hypocenter of the nuclear explosion showed microencephaly and mental retardation, and one was a mongoloid type (22).

It has been the general medical practice to avoid using radiographic procedures for pregnant women except for pelvimetry or a flat film of the abdomen when indicated. Because of the possibility that the radiation doses during fluoroscopic procedures might affect the embryo during the second to seventh week, Russell (20) recommends that women of the childbearing age should be subjected to such procedures only within two weeks after menses. This would avoid exposure of the embryo in cases of unsuspected pregnancies.

Tumor induction:—The first epidermoid carcinoma occurring in the grossly overexposed skin of a pioneer in the field of radiology was reported in 1902 (23). Since then there has been ever-increasing experimental evidence that exposure of almost any tissue in the body can lead to malignant change if suitable conditions of irradiation are used. Such exposure conditions usually involve large doses of radiation, often protracted over a long period of

small fraction of the population was exposed occupationally or in the course of medical radiography.

Realizing that exposure to radiation is inevitable in our civilization and indeed can benefit mankind as in the case of medical radiography and atomic energy, geneticists have attempted to agree on a radiation dose to which large populations can be exposed without causing excessive hardship to society in future generations. For the general population, the committees of the British Medical Research Council (1) and the National Academy of Science (2) agree in essence that gonadal exposures should be kept to a minimum and should not exceed approximately twice background or 10 r during the reproductive life of the individual. In the case of the small fraction of the population occupationally exposed, they recommend that the total dose per individual should not be greater than 50 r. In considering the maximum permissible genetic dose, the International Commission of Radiological Protection (17) recommends that the whole population should not receive from all sources other than natural background more than 5 r (from age zero to 30) plus the lowest practical contribution from medical exposure.[3]

As has been pointed out, much of the unnecessary gonadal exposure in the past can be reduced by improved radiologic techniques. However, we must not lose sight of the fact that the risk to each individual and his descendants is so small that no one with a potentially serious disease should be denied the benefits of diagnostic radiology for genetic reasons. We must also remember that radiation is only one of many possible mutagenic agents in our environment. We know almost nothing about the action of the many chemicals in the air we breathe, the food we eat, and the medicines we use.

Somatic Effects

Since the first recorded skin burn caused by exposure to ionizing radiation in 1896 (19), it has been known that radiation in sufficient dosage can damage human tissue. In the past 15 or 20 years, it has become evident that radiation doses which cause no obvious immediate effect can, nevertheless, be responsible for late changes in exposed tissues. Such changes occurring many years later may ultimately lead to severe injury or to the death of the exposed individual. In the case of repeated exposure over many years it has been known that malignant degeneration or other undesirable changes of tissue might result. In these cases the total accumulated dose was invariably high, usually amounting to thousands of roentgens. Within the past ten years, it has become apparent that small doses of radiation in the range of 100 to 200 r

years for thymic enlargement with smaller doses of x-rays through smaller ports than those formerly used (32). He concludes that the difference of leukemia rates in the various studies may be explained on the basis of the volume of tissue irradiated and the part of the body treated as well as on the dose. In contrast to the contradictory observations on leukemia incidence, the increased frequency of thyroid cancer is substantiated in all but one prospective study and in numerous retrospective studies (33). The lowest dose associated with thyroid cancer was 100 r (30).

The third group of persons studied after radiation exposure consists of a group of patients with ankylosing spondylitis given x-ray treatments to the spine. This study, reported by Court-Brown & Doll (34), is of great interest because the radiation doses are known accurately and the number of cases of leukemia is sufficiently large to attempt to relate the dose to the incideoce of disease. Of 32 confirmed and five probable cases of leukemia, all but one were acute in nature or, if chronic, then of the myeloid type. There is a clear relationship between dose and leukemia incidence in patients receiving more than 500 r to the spine. The group with the greatest exposure (2750 r) had about a 35-fold increase in the incidence of leukemia over that in the general male population. Since only two cases of leukemia received less than 500 r to the spine (and one of these received additional extra spinal treatments), one cannot be certain that the same dose relationship exists at low-dose levels. Nevertheless, the authors point out that extrapolation of the curve of dose versus leukemia incidence to the low-dose range suggests that the response may be non-threshold. One criticism of this hypothesis is that nothing is known of the leukemia incidence in untreated patients suffering from ankylosing spondylitis, a disease with a strong hereditary component. Indeed, there is a suggestion that the leukemia incidence in persons suffering from rheumatism is higher than it is in the general population (35).

The fourth study of leukemia incidence in the irradiated population of Hiroshima, has been reported by Heyssel *et al.* (36). Here, again, there is a clear relationship between the estimated dose or the distance from the hypocenter beneath the atomic explosions and the leukemia incidence. How far down the dose scale the correlation holds is not certain, but the authors state that they believe an increased incidence occurs in persons receiving the equivalent of 50 to 100 r. It must be pointed out that considerable uncertainty exists as to the exact location of the persons at the time of exposure and the reliability of even the best current dose estimates. If, as seems probable, the dose estimates are of the right order of magnitude, then the leukemia incidence per roentgen of total body exposure turns out to be about one to two cases per roentgen (RAD) per year after exposure per one million people exposed; at least, this is true in the dose range above 100 r and holds for at least 10 to 15 years after exposure (37). It is of interest that children appear to be more susceptible to the leukemogenic action of radiation than are adults and that chronic lymphatic leukemia was rarely observed (36).

So far we have been speaking of leukemia incidence in persons receiving

time, and delivered to a small volume of tissue. We are interested here, however, io the carcinogenic action of radiation doses used io medical practice aod believed uotil recently to be perfectly safe. Only studies of leukemia aod thyroid cancer will be discussed as they provide the only data available relating dose to cancer iocideoce in man.

Epidemiological surveys of the incidence of leukemia and thyroid cancer after radiation exposure have been made of five types of human population groups. The first statistically valid study was that of Heosbaw & Hawkins (24), who reported in 1944 that the iocidence of leukemia deaths in American physicians was almost double that in the general male populatioo. Subsequent surveys of deaths amoog radiologists in this country have coofirmed these observatioos, but in receot years the leukemia iocideoce among radiologists seems to be decreasiog (25). A comparable study of British radiologists revealed no increase io leukemia incidence sioce 1921 (26). The difference io leukemia frequency in these two groups similarly exposed has been attributed to better radiation protection measures used in Great Britain, The American studies are valuable in showing that repeated exposure to radiation, primarily to the haod, arms, and upper torso (depending upon the protective measures used), can lead to the development of leukemia. They are of no value in establishiog the quantitative relationship of dose to leukemia incidence as there are no data concerning exposure levels.

A study of children given x-ray therapy in infancy for thymic enlargement reported by Simpson & Hempelmann (27) revealed an increased incideoce of leukemia and other forms of cancer, especially thyroid cancer, over that expected to occur in the general population of the same age or in untreated siblings of the treated children. The number of cases of leukemia was ten times that expected, whereas 100 times as maoy cases of thyroid malignancy were fouod. This study was of particular interest because some cases of leukemia were observed io children who had received between 100 aod 200 r of x-rays, a dose formerly considered to be completely innocuous. Io a group of 2400 children so treated (28), the leukemia iocideoce did oot show an obvious dose dependence. For example, four cases were observed in 1050 children receiviog less than 200 r and five cases in 1025 childreo receiving more than 200 r aod usually less than 600. An increased number of leukemia cases was also observed in 75 children treated for pertussis and io about 1100 children given x-ray treatments to the head aod neck (29).

The role of radiation io the developmeot of leukemia in these children is difficult to differentiate from that of the medical conditioo for which the treatmeot was given. It is quite clear that there are available no cootrol groups comparable in all ways to the children selected for treatmeot except for radiation exposure. Other surveys of children given x-ray treatments have confirmed the high iocidence of thyroid cancer but have not showo more thao the expected number of cases of leukemia (30, 31). Ao author of the original study of children mentioned above also has not found ao increased frequency of malignancy in a second group of 1350 children treated in receot

Life shortening.—Experimental studies chiefly on rodents have established the fact that exposure of animals to substantial doses of total body radiation causes a shortening of life span in the species used (41). When doses approach the acute lethal dose, up to 50 per cent reduction of life span is observed. With doses less than 200 r, it is difficult to demonstrate significant life shortening. In this range, the suggested upper limit of 1 to 1.5 per cent shortening of total life span per 100 r compares favorably with the life-shortening of 11 per cent per 1000 r produced by protracted exposures. Much of this effect is due to what seems to be a hastening of the natural aging processes with the result that diseases noted primarily in older animals appear earlier in the life of the irradiated individuals (25). There is little species variation with regard to this effect, suggesting that it is non-specific in nature. In certain animal strains with a high incidence of leukemia or mammary carcinomas, radiation exposure hastens the time of appearance of these diseases. In these cases the effect appears to be linked to the genetic constitution of the animals (25).

The only published attempts to study life-shortening in man after radiation exposure have dealt with radiologists. When corrected for the difference in age distribution of radiologists and other specialists, the most recent studies in the United States and Great Britain do not disclose any evidence of life-shortening in physicians practicing radiology (26, 42). In an older study of the mortality of physicians in the years 1938–1942 (43), the mortality of radiologists was higher than that of other physicians but the difference was only of borderline significance because of small sample size. If expressed in terms of life expectation, the life-shortening in this study amounted to 1 to 3 years, with leukemia deaths accounting for only one-quarter of the effect(25).

The absence of significant life-shortening of the relatively heavily exposed radiologists indicates that exposure of patients during medical radiography is not likely to present any serious danger to the general population in this respect.

Fertility.—The gonads are among the most radiosensitive tissues in the body. Exposure to as little as 5 to 25 r causes microscopic evidence of tissue damage (44). Unlike most radiobiologic phenomena, a greater effect on the germinal epithelium occurs when a given dose is protracted rather than delivered in a brief period (45). Thus, in recent experiments on dogs, a single dose approximating the LD_{50} was needed to cause almost complete temporary sterility and an LD_{100} was needed to sterilize permanently. In contrast, exposure of dogs to 3 r per week for a year (total dose 156 r) caused 80 per cent of exposed dogs to be sterile and 20 per cent to have reduced sperm counts (45).

In man, a single dose of 150 r may produce temporary subfertility or sterility in many men and women, especially in cases of borderline fertility, whereas 500 to 800 r are required to produce permanent sterility (46). Limited experience with groups of persons exposed to substantial doses of radiation

radiation exposure in excess of that used in most diagnostic radiologic procedures. Recently, Stewart, Webb & Hewitt (38) reported a retrospective study of the history of *in utero* exposure of children dying of leukemia and other forms of cancer. This study was compared with that obtained from control children selected from the birth registry. It was found that twice as many children with malignant disease had been irradiated *in utero* as had controls (13.7 per cent versus 7.2 per cent). No definitive conclusions as to the role of radiation in the development of malignancy in these children could be reached from this study for two reasons. First, it is impossible to differentiate between the role of the procedure and the medical condition for which the exposure was given in the development of leukemia. Secondly, other studies using different methods for selecting controls do not substantiate these observations (39). An unpublished prospective study has been made in Great Britain of almost 40,000 live-born children whose mothers had abdominal radiographs during pregnancy (40). The results of this study do not disclose an excess number of cases of leukemia over that expected in a group of children of this size and with this age distribution. Other studies now under way should soon answer the question as to whether or not small doses of x-ray of the magnitude used in diagnostic radiology are leukemogenic for the human fetus.

In summarizing the available information for man, it may be said that there is no question about the leukemogenic action of radiation in persons receiving more than 100 r to a large portion of their bodies. The absolute number of cases of leukemia in which radiation may have played a role is small, only 226 cases (including the Japanese) having been published to date in the literature (37). In the range of 100 r (or its equivalent) and above, the leukemogenic effect increases with increasing doses. In this range, the risk of developing leukemia in the exposed population is approximately one to two cases per roentgen per year following exposure per one million exposed people. There is no information to indicate whether or not doses below 100 r are leukemogenic.

It also seems likely that children are more sensitive to the leukemogenic action of radiation than adults. Under certain conditions of exposure, the thyroid of children may be more sensitive to the carcinogenic action of radiation than are the blood-forming tissues. This is in marked contrast to the adult thyroid which appears to be very radiation-resistant.

One may conclude that the risk of developing malignant disease from the usual medical radiographic procedures is very slight if, indeed, there is a risk. Perhaps an exception to this is repeated fluoroscopic examination of babies which can cause substantial exposures of a large part of the body. X-ray treatment of benign conditions in children increases the risk of developing thyroid cancer and probably leukemia. Even here the risk to the individual is small in absolute terms but becomes important when large groups are so treated.

reproductive age should be confined if possible to within two weeks after menses. Doses of x-rays of the magnitude delivered during a fluoroscopic examination have been shown to produce developmental abnormalities in animals.

Other somatic effects of radiation such as shortening of life span or decreased fertility do not appear to present a serious problem to the population as a result of medical procedures.

LITERATURE CITED

1. British Medical Council, *The Hazards to Man of Nuclear and Allied Radiation* (Her Majesty's Stationery Office, London, Engl., 1956)
2. Summary Report, "The Biological Effects of Atomic Radiation," *Natl. Acad. Sci.—Natl. Research Council*, (Washington, D. C., 1956)
3. Buscke, F., and Parker, F. M., *J. Pediat.*, 21, 524–33 (1942)
4. Norwood, W. D., Healy, J. W., Donaldson, E. E., Roesch, W. C., and Kirklin, C. W., *Am. J. Roentgenol.*, 82, 1081–97 (1959)
5. Laughlin, J. S., and Pullman, I., "The Gonadal Dose from the Medical Use of X-rays (Prelim. Rept.)," *Natl. Acad. Sci.—Natl. Research Council* (March, 1958)
6. Muller, H. J., *Science*, 66, 84–87 (1927)
7. *Report of the United Nations Scientific Committee on the Effects of Atomic Radiation* (General Assembly, Official Records, 13th Session. Suppl. *No. 17 (A/3838)*, New York, N. Y., 1958)
8. Russell, W. L., *Am. Naturalist*, *90*, Suppl., 69–80 (1956)
9. Russell, W. L., Russell, L. B., and Kelly, E. M., *Science*, 128, 1546–50 (1958)
10. Russell, W. L. (Personal communication)
11. Russell, W. L., *Proc. Natl. Acad. of Sci.* (U. S.), 43, 324–29 (1957)
12. Summary Report, "The Biological Effects of Atomic Radiation," *Natl. Acad. Sci.—Natl. Research Council* (Washington, D. C., 1960)
13. Macht, S. H., and Lawrence, D. S., *Am. J. Roentgenol.*, 73, 442–66 (1955)
14. Crow, J. F., *Am. J. Roentgenol.*, 73, 461–71 (1955)
15. Stevenson, A. C., *Radiation Research*, Suppl., 1, 306–25 (1959)
16. Muller, H. J., in Hollaender, A., *Radiation Biology*, I, 351–453 (McGraw-Hill Book Co., New York, N. Y., 1954)
17. *Recommendations of the International Commission on Radiation Protection* (Pergamon Press, Inc., New York, N. Y., 1959)
18. Russell, W. L., in Hollaender, A., *Radiation Biology*, I, 825–58 (McGraw-Hill Book Co., New York, N. Y., 1954)
19. Marcuse, W., *Deut. med. Wochschr.*, 22, 481–83, 681–82 (1896)
20. Russell, L. B., in Hollaender, A., *Radiation Biology*, I, 861–918 (McGraw-Hill Book Co., New York, N. Y., 1954)
21. Rugh, R., in Errera, M., and Forssberg, A., *Mechanisms in Radiobiology*, II, (Academic Press, Inc., New York, N. Y., 1960)
22. Plummer, G., *Pediatrics*, 10, 687–93 (1952)
23. Frieben, A., *Fortschr. Gebiete Röntgenstrahlen*, 6, 106 (1902)
24. Henshaw, P. S., and Hawkins, J. W., *J. Natl. Cancer Inst.*, 4, 339–46 (1944)
25. Report of the Subcommittee on Long Term Effects of Ionizing Radiation from External Sources, *Natl. Acad Sci.—Natl. Research Council*, (Washington, D. C., in press)
26. Court-Brown, W. M., and Doll, R., *Brit. Med. J.*, II, 181–87 (1958)
27. Simpson, C. L., and Hempelmann, L. H., *Cancer*, 10, 42–56 (1957)
28. Simpson, C. L., *Radiation Biology and Cancer*, 336–46 (Univ. of Texas Press, Austin, Texas, 1959)
29. Murray, R., Heckel, P., and Hempelmann, L. H., *New Engl. J. Med.*, 261, 585–89 (1959)
30. Saenger, E. L., Silverman, F. N., Sterling, T. D., and Turner, M. E., *Radiology*, 74, 889–904 (1960)
31. Latourette, H. B., and Hodges, F. J., *Am. J. Roentgenol.*, 82, 667–77 (1959)
32. Hempelmann, L. H. (Unpublished data)
33. Rooney, D. R., and Powell, F. W., *J. Am. Med. Assoc.*, 169, 1–4 (1959)

approaching the lethal dose did not indicate a serious permanent effect on fertility (46). Alsn, two large-scale surveys of the offspring of radiologists previnusly mentioned (13, 14) did not indicate a decrease in fertility.

These studies are reassuring with respect to the possihle effect on fertility of radiation frnm medical radiologic practice. Despite the apparent unaltered fertility of radiologists, the studies in dogs showing the surprising effect of small protracted doses of radiation would seem to call fnr caution on the part of people occupatinnally exposed to x-rays over a long period of time.

Summary and Conclusions

As the radiation exposure of the world's populatinn increases, additional exposure fnr medical purposes can pose a distinct threat to tbe health and well-being of mankind. As one of the known mutagenic agents in nur environment, there is every reason to believe that even tbe small doses of radiation used in diagnostic radiology will contrihute mutant genes to the gene "pool" of the total population. Since all mutations are deleterinus to some degree and since those occurring spontaneously contribute substantially to the burden of disease in society, it is important to minimize gonadal exposure during the reproductive life of man. It has been recommended tbat the average gonadal dose of everyone in our population should never exceed 5 to 10 r during the first 30 years of life. Medical exposure can be decreased by avoiding unnecessary radiographic procedures, by use of good radiologic technique, and by shielding the gonads wben necessary. Because the risk to each individual and his offspring is small, no patient with a potentially serious disease sbould be denied the henefits of radiographic procedures on tbe basis of possible genetic damage.

Altbough radiation exposure is known to be leukemogenic and carcinogenic, medical exposures do not seem to present a serious hazard to the population unless grossly overused. (A possible exceptinn is found in the case of children exposed to radiatinn prenatally.) Exposure of tbe entire body or a large part thereof to doses in excess of 100 r increases the incidence of leukemia. Exposure nf the neck and chest of children to doses of this magnitude is also associated with thyroid neoplasia. Exposures of this magnitude are unusual in diagnostic radiology except perhaps during repeated flunroscopic examination, especially nf children. Such doses are often emplnyed in the therapy of benign conditions. Even with exposures of a large part of the body to doses exceeding 100 r, the risk of developing leukemia is small (of the order of one to two per million per roentgen per person per year after exposure). An excess in the number of cases of leukemia becomes evident only when large groups are so exposed. There is no evidence in man to indicate whether or not doses below 100 r are leukemogenic.

Since the developing embryo and fetus is exceedingly sensitive, care must be used in avoiding abdominal exposure of pregnant mothers. Since the period of greatest sensitivity occurs wben pregnancy may be unsuspected (2 to 6 weeks), fluoroscopic procedures involving the abdomen of wnmen in the

NEOPLASTIC DISEASE: TUMOR METABOLISM[1,2]

By Harris Busch, M.D., Ph.D.,[3] and Joseph R. Davis, M.D.[4]
Departments of Pharmacology and Biochemistry, Baylor University College of Medicine, Houston, Texas

"What's past is prologue"[5]

The biochemical approach to cancer chemotherapy is based upon the recognition of the fact that cellular reproduction is essentially a biosynthetic process and that, in some way, the biosynthetic activities of neoplastic cells are different from those of non-tumor cells. There is an enormous hiatus between the cytological description of cell structure and function and the laborious endeavors of biochemists to define the fundamental mechanisms involved in functions of cells and their substructures. If it can be postulated that the function of the cancer cell is growth, abnormal growth to be sure, the necessity of intensive study of all related biosynthetic reactions becomes clear. It is not now possible to postulate whether the chief difference between cancer cells and other cells lies in biosynthesis of proteins, carbohydrates, lipids, nucleic acids, or any other cellular entity such as the nuclear or cellular membranes. The present clues to the solution of the cancer problem lie in the demonstrated histological characteristics of cancer cells, i.e., the more intensive staining of the nucleus and the multiplicity of division of individual cancer cells. It is because these clues suggest that the primary defect in the neoplastic cell is in the cell nucleus that biochemical studies in recent years have tended more and more to emphasize the biosynthetic activities of the nucleus and the role of nuclear materials in transmission of genetic changes. The most challenging problems in cancer chemotherapy at present are the definition and exploitation of the differences in nuclear constituents and function of neoplastic cells and other cells.

The object of this review will be to present the highlights of progress in the biochemical understanding of these areas. Needless to state, the limitations of space are such that many important contributions will be omitted, but it is hoped that references to pertinent reviews will compensate in part for the omissions. In addition, some of the salient advances in special areas of the biochemistry of the cancer cell will be indicated.

[1] The survey of the literature pertaining to this review was concluded in July, 1960.

[2] The following abbreviations will be used: DNA (deoxyribonucleic acid); RNA (ribonucleic acid).

[3] The original studies reported in this review were supported in part by grants from the United States Public Health Service, The American Cancer Society, and the Jane Coffin Childs Fund.

[4] Post-doctoral Fellow of The American Cancer Society.

[5] Shakespeare. The Tempest II.

34. Court-Brown, W. M., and Doll, R., *Med. Research Council Rept.*, No. 295, (Her Majesty's Stationery Office, London, Engl., 1957)
35. Abbatt, J. D., and Lea, A. J., *Lancet*, II, 880 (1958)
36. Heyssel, R., Brill, A. B., Woodbury, L., Nishimura, E. T., Ghose, T., and Hoshino, T., *Blood*, 15, 313–31 (1960)
37. Cronkite, E. P., Maloney, W., and Bond, V. P., *Am. J. Med.*, 38, 673–82 (1960)
38. Stewart, A., Webb, J., and Hewitt, J., *Brit. Med. J.*, I, 1495–1508 (1958)
39. Hempelmann, L. H., *Cancer Research*, 20, 18–27 (1960)
40. Court-Brown, W. M., and Doll, R., *Proc. Roy. Soc. Med.* (In press)
41. Blair, H. A., *Proc. Intern. Conf. Peaceful Uses Atomic Energy*, 11, 118–20 (1956)
42. Seltser, R., and Sartwell, P. E., *J. Am. Med. Assoc.*, 166, 585–87 (1958)
43. Dublin, L. I., and Spiegelman, M., *J. Am. Med. Assoc.*, 137, 1211–15 (1948)
44. Oakberg, E., *Radiation Research*, 2, 369–91 (1955)
45. Cassarett, G. W., and Hursh, J. B., *Proc. Intern. Conf. Peaceful Uses Atomic Energy*, 11, 184–88 (1956)
46. Cassarett, G. W., *Univ. of Rochester Rept.*, No. 441 (1956)

tissues must await methods for separation, without accompanying degradation, of the population of nucleic acids present in such tissues plus methods for the determination of nucleotide sequences.

Pictures of chromosomes (13) reveal the very remarkable intermingling of protein and nucleic acids in those structures. It hardly seems likely that the DNA molecules along this type of chain are the same, much less the types of DNA from chromosome to chromosome. Fractionation of DNA has been attempted with some success by Bendich *et al.* (14) by means of substituted cellulose columns (Epichlorohydrintriethanolamine cellulose). Recent studies have tended to cast some doubt on the state of the original preparations from which the chromatograms were derived (15). Studies on base composition of the products have not indicated remarkable differences in the eluted fractions (10, 11, 12). Despite objections, this method is thus far the only one which has shown significant promise in the fractionation of the nucleic acids.

At the present time, no significant chemical differences have been established between the nucleic acids of neoplastic and other tissues. Petrakis *et al.* (16) have iodicated that the Sternberg-Reed cells of Hodgkin's disease have an increased content of DNA over the diploid level as measured by Feulgen cytophotometry. Other studies indicating high levels of DNA in the nuclei of neoplastic cells have been reported by Giordano *et al.* (17) and by Scarloto & Muller (18).

Sequence analysis of the nucleic acids.—There must be a wide variety of linkages in nucleic acids since the "nearest neighbor" technique has shown the presence in nucleic acids of every type of predictable nucleotide-to-nucleotide linkage (19). Some success has been obtained in the endeavor to develop methods for sequence analysis by a variety of systems (20, 21). However, such analytical methods are valid only when the starting material is pure. For such studies, the purest starting materials at present may be obtained from viral sources, but a great need in the biochemistry of tumors is for the development of methods for fractionation of the chromosomes, separation of the component DNA molecules, and study of their structure.

Synthesis of DNA.—Special mechanisms for the biosynthesis of nucleic acid (22) have not been found in tumors. Smith *et al.* (23), have reported that DNA synthesis in Hela cells occurred during interphase in two time periods, one shortly before and another shortly after the process of cell division. Several groups have reported the "superadditive" incorporation of the deoxynucleotides of thymine, adenine, cytosine, and guanine into DNA by soluble enzyme systems found in lymphoblasts obtained from mice and in extracts of mouse ascites tumors. "Primer" DNA was required for this synthesis as well as Mg^{++} and ATP. Smellie *et al.* (24) have indicated that biosynthesis of DNA in tumor extracts proceeded at a rate sufficient to double the DNA content of the system in approximately 5 to 6 hr. Extracts of

Isolation of Nuclei

In studies on nuclear metabolism, proper isolation of the nuclei is a fundamental necessity. Recent developments include the method of Chauveau, Moulé & Rouillier (1), who sedimented highly purified nuclei from the liver through a dense sucrose solution (2.2 M) in a force field of 40,000×g. Modification of their method in this laboratory permitted isolation of nuclei from neoplastic cells in which the purity of the nuclei is 99 per cent by volume and 88 to 99 per cent by differential count of isolated nuclei (2). With this method nuclei in highly pure form have been obtained from the Walker, Flexner-Jobling and the Jensen tumor of the rat, sarcoma 180 of the mouse, and a human malignant melanoma. Increasingly satisfactory methods for preparation of nuclei of neoplastic cells have pointed up the possibility of purification of nuclear components, such as nucleoli, chromosomes, nuclear membranes, centrosomes, and other nuclear fractions.

Structure of Deoxyribonucleoprotein

The structure of the deoxyribonucleoprotein complex has been investigated mainly by physical methods. Zubay & Doty (3) have found the molecular weight of the complex obtained from calf thymus to be 18.5 million. Of the molecular weight, approximately 8 million is contributed by deoxyribonucleic acid; the other 10.5 million is contributed by protein. They (3) suggested that the protein is evenly distributed along the DNA chain, possibly in the "deep groove" of the nucleic acid. The nuclear protein molecules (m.w. 30,000), may either add to the genetic potentialities of the nucleic acid or suppress specific genetic potentialities. There is good agreement (4) that the structure of DNA is a double-stranded α-helix in the nuclei of most species (5, 6). The x-ray diffraction pictures of the DNA have been found to be essentially the same when the DNA was obtained from viral, bacteriological, and animal sources as well as from tumors. However, DNA from one phage (ϕX-174) has only a single strand (7). Electron microscopic procedures (8) have shown that depending upon the salt concentration, DNA has either a linear or a globular structure.

Another method for characterizing nucleic acids is the determination of the base content. Recently (9 to 12), RNA and DNA were isolated from a variety of tissues of chicken and mouse and were analyzed for their base content. Significant differences were not found in base composition between the respective nucleic acids of the normal and neoplastic tissues. However, Kleinschmidt (9) states:

> If the nucleic acids play their role as prototypes, templates and carriers of coded information, then of necessity they must somehow differ. It is reasonable to assume that the differences lie in the sequential arrangement of the purine and pyrimidine nucleotides along the nucleic acid chain. Elucidation of the differences of nucleic acids to explain such biological differences as are exhibited by normal and neoplastic

the cells, the content of thymidylate synthetase and deoxycytidylate hydroxymethylase increased markedly. When the virus did not contain hydroxymethylcytosine, only the content of the thymidylate synthetase was found to increase. The virus would appear to contain the templates or pretemplates for the biosynthesis of these special enzymes. Many of the enzyme potentialities of cells would then be inherent in the DNA and the other chromosomal components, and tbese may be reflected in the development of specific proteins and enzymes which define the characteristics of the individual and of the species.

Biosynthesis of the precursors of DNA.—Many studies have been carried out on formation and catabolism of the precursors of DNA including the purine bases, adenine and guanine and the pyrimidine bases, thymine and cytosine, as well as the sugar moiety, deoxyribose. There is no evidence that the biosynthetic pathways differ in neoplastic cells as compared with other cells (33, 34), providing that the conditions employed provide adequate precursors and proper sources of energy for the reactions. Despite the fact that significant differences between tumors and other tissues in these reactions have not been demonstrated, the formation of the purines and pyrimidines have been the chief recent target of antineoplastic agents developed. The goal of analogue therapy has been either to block the formation of nucleic acids in neoplastic tissues by preventing nucleotide formation, or by direct incorporation of the abnormal base into the nucleic acid to interfere with the function of the nucleic acids. In general, these attempts have met with only very limited success since it is apparent that the attack is not directed against specific metabolic characteristics of tumors. The hope in development of agents such as 5-fluorouracil was that neoplastic cells might have a more rapid uptake of the antitumor agent since the uptake of uracil-2-C^{14} into tumors was found to be greater than uptake into other tissues (35, 36). Unfortunately, the rate of uptake of 5-fluorouracil by human tumors does not seem to be sufficiently greater than that of other tissues to enable 5-fluorouracil to be a selectively toxic agent for human neoplasms.

Control of DNA biosynthesis.—Metabolic controls may be extracellular, such as hormones, may, on the surface of the cell, be related to permeability, or may be intracellular controls. Examples of the latter are "feedback controls" of purine and pyrimidine metabolism. Yates & Pardee (37) have reported that the concentration of uracil controlled the formation of enzymes required for the biosynthesis of orotic acid, a precursor of uracil. This type of "feed-back" suppression of biosynthesis is related to the presence of an end product in a concentration sufficient to suppress enzyme formation. Another "feed-back control" is that in which the activity of an enzyme involved in a biosynthetic chain is suppressed by an end product. Reverse competitive inhibition has been demonstrated for a number of steps in the biosynthetic pathways by formed products. One of the more well-defined initial reactions of purine nucleotide biosynthesis is the formation of phos-

ascites tumor cells prepared by sonic treatment incorporated thymidine into DNA, either as a terminal nucleotide, or as part of a polymeric extension of DNA.

These studies on tumors are an extension of studies of Kornberg *et al.*, on the mechanisms of biosynthesis of DNA in bacteria and the studies on RNA biosynthesis of Ochoa *et al.* The basic essentials of the system for biosynthesis of DNA in cell-free systems included the availability of the deoxyribotides of adenine, guanine, cytosine, and thymine, special enzymes (the DNA or nucleotide polymerase), Mg^{++}, and "primer" DNA. The polymerase was purified 2000-fold by Bessman *et al.* (25). The base composition of the product of the reaction conformed to theory (5, 6), i.e., the product contained equal numbers of adenine and thymine residues and equal numbers of cytosine and guanine residues. The ratio of the A-T pairs and the G-C pairs was dependent upon the particular primer used. The experiment indicated that replication of DNA was possible by means of the "polymerase" enzyme and the soluble constituents added to the system.

DNA content of special chromosomes.—One of the needs in modern cancer biology and biochemistry is to determine the nature of the DNA in the complex chromosomal structures. Hsu and others (26, 27, 28) have developed methods for the study of chromosomes by arrest of cell division and by spreading of the chromatids by means of hypotonic solutions. Remarkable findings have been made which indicate extensive changes in the chromosome patterns of neoplastic cells as compared with others. A common pattern of these changes has not yet been found, i.e., some of the chromosomes of the neoplastic cells are larger than those of the non-tumor cells, others are smaller, still others are distorted. It would be of interest to determine whether one or more of these chromosomes is the same as or different from the corresponding chromosomes of non-tumor tissues. As indicated above, the development of methods for the isolation of chromosomes is an important area of modern cancer research.

Function of DNA.—The first clear-cut evidence that DNA contains either the templates for enzyme synthesis or the initiating factors for development of such templates, has emerged from studies made by Cohen *et al.* (29 to 32). They determined the enzyme content of bacteria before and after infection with bacteriophages containing DNA as their chief nucleic acid. Some of these bacteriophages contained a specific nucleotide, 5-hydroxymethyldeoxycytidylic acid. The enzyme for the formation of this nucleotide from deoxycytidylic acid, i.e., deoxycytidylate hydroxymethylase, was not found in the uninfected bacteria or in the bacteriophage, but in the infected cells. Since the virus did not contain any deoxycytidylic hydroxymethylase, formation in the cell must have been induced by the virus. When thymine-requiring bacterial mutants were studied, their content of thymidylate synthetase, which catalyzes formation of thymidine, was found to be virtually undetectable. Depending upon the type of bacteriophage used to infect

is soluble in aqueous phenol but not soluble in water (RNA-R). In neoplastic tissues studied, there was a very low concentration of the RNA-R, i.e., 5 to 10 per cent of the extractable RNA. In non-tumor tissues such as liver, the concentration was higher, 15 per cent or more of the extractable RNA. The turnover of the RNA-P was higher than that of the RNA-R as measured by the uptake of P^{32} and this accounted in part for the greater overall turnover of the RNA of the tumor. A partial explanation of the relatively high guanylic acid content of the RNA of tumors (55, 56) was that the guanylic acid content of RNA-P was high while that of RNA-R was low. This high level of guanylic acid in RNA has not been a universal finding since Kleinschmidt (9) reported that the base composition and the base ratios were not markedly different in the Rous sarcoma and the mouse ascites tumor from the values obtained in the non-tumor tissues studied.

Nucleolar RNA.—The nuclear RNA, of which a very large portion is in the nucleolus, accounts for much of the cellular RNA in all tissues and even more in tumors (57). Indeed, Caspersson & Santesson (57) have stated that increase in nucleolar mass is a practically constant feature of malignant disease. More recent evidence for differences in the size of the nucleoli of neoplastic cells and other cells has been obtained by Stenram (58). In a statistical analysis of the volume of nucleoli of livers and hepatomas of rats fed diets rich or poor in protein, Stenram noted that in the tumor the nucleolar volume was approximately 3.8 μ^3. The nucleoli of the normal liver of the animal fed a 25 per cent casein diet was 1.8 μ^3; this volume was increased to 3.3 μ^3 on a non-protein diet. The nucleolar volume of the tumor and of other tissues did not change in response to the diet. Evidence for the complex distribution of the RNA in the nucleus has been provided by Love & Liles (59) who reported RNA in the nucleolus, parachromatin, chromosomes, and perichromosomal regions. Further evidence for the complexity of the nucleolar structure has provoked the suggestion that the nucleolus may have an outer core of metabolically active RNA and an inner core that is metabolically more inert (60). The primary sources of evidence regarding the nucleolus have been microscopic studies or studies on non-mammalian species. Such studies have led to much speculation about the role of the nucleolus as a possible genetic locus, a site of RNA synthesis, a site at which RNA synthesized on different genes tends to agglomerate (60), or a site of biosynthesis of nuclear or other proteins (61).

Metabolism of RNA.—Significant differences between tumors and other tissues have been reported in studies on the incorporation of labeled uracil into RNA (35, 36, 62). Although such studies have suggested that tumors utilize nucleic acid precursors *in vivo* to a greater extent than other non-growing non-tumor tissues, the uptake of the precursor into intestinal RNA exceeded that of the tumor (*vide infra*). As indicated by a number of authors (see above), there is no other evidence for a difference in either the formation or utilization of the bases which are found in either DNA or RNA of tumors.

phoribosylamine from phosphoribosylpyrophosphate and glutamine. This reaction was inhibited by a number of compounds including pyrophospborylated purine nucleotides as well as the nucleosidemonophosphates. Wyngaarden & Ashton (38) have suggested that a control at this level of purine biosynthesis would provide a system for rapid biosynthetic control to insure orderly synthesis and to maintain constancy of concentration of purine ribonucleotides and deoxyribonucleotides. That such feedback controls are operative in neoplastic cells has recently become evident from the studies of Bresnick (39) who found that a number of pyrimidines and analogues suppressed the activity of dihydro-orotase and ureidosuccinate synthetase. In discussing these phenomena and their relationship to the problem of neoplasia, Potter (40) has suggested that deletion of feedback controls througb the action of carcinogens is related to the development of neoplasia.

Ribonucleic Acids (RNA)

Microsomal RNA.—Although the functions of the cytoplasmic ribonucleic acids (RNA) have been partially clarified, functions of nuclear RNA are less well defined. The intimate relationship of microsomal RNA to protein synthesis has been established by recent studies (41 to 44), which have shown by immunological and other metbods that the synthesis of proteins such as albumin and pancreatic enzymes occurs in the microsomal particle. Attempts to purify the templates responsible for these syntheses by solubilizing the attached lipids have led to the isolation from microsomes of very active particles rich in ribonucleic acids which have been named "ribosomes" (44, 45, 46).

s-RNA.—Protein formation is a multistage process of which the last step would seem to be linking of "activated" amino acids on the surface of microsomal ribonucleoproteins. Berg (47) has shown that amino acids are initially coupled in anhydride linkage to adenylic acids to form aminoacyladenylates. From these "activated" structures, the amino acid is transferred to a low molecular weight RNA of the cytoplasmic sap, i.e., the s-RNA. Brachet (48) and Sirlin (49) have indicated tbat the s-RNA contains 30 to 120 nucleotide residues and hence has a molecular weight of 20,000 to 60,000 while the microsomal RNA contains approximately 5000 residues and has a molecular weight of approximately 2,000,000. Recently obtained evidence (50, 51) suggests that the amino acid transferring RNA is composed of a number of molecular species of which one is specific for each amino acid. Similar specificity may exist for the microsomal RNA and there may be as many types of RNA in the cell as there are proteins. Studies on the fractionation of the microsomal RNA are in progress (52) but have not yet reached even the state of definition now existing for the s-RNA.

RNA of neoplastic tissues.—Using the method devised by Kirby (53), Sibitani *et al.* (54) have found two types of RNA in cells, one that is soluble in aqueous phenol and then extractable into water (RNA-P) and one that

Nuclear Proteins

Recent reports (61, 71, 72, 73) have indicated a rapid rate of biosynthesis of nuclear proteins in neoplastic tissues. Comparative studies on a variety of neoplastic tissues, growing non-tumor tissues and non-growing, non-tumor tissues have suggested that the nuclear proteins biosynthesized in the neoplastic cells may be different entities from those synthesized in other tissues (61). These findings have been made possible by the use of radioactive techniques and by the rapid development of refined methods for the isolation and purification of nuclear proteins (74, 75) which have been based upon techniques for fractionation of other proteins (76).

The isolation, identification, and determination of function of the nuclear proteins of neoplastic cells are part of the general problem related to corresponding studies on these proteins in non-tumor cells (77). Perhaps the greatest need confronting biochemists working in this area is the isolation of these proteins in highly purified form. The first problem of initially isolating nuclei in a highly purified form has now been largely solved (1, 2). The studies of Butler's group (78, 79) have established the importance of adequate use of modern methods for characterization of the nuclear proteins, such as sedimentation behavior, solubility, amino acid content, and end-group analysis (80, 81).

Number of nuclear proteins.—Early reports established the presence of a minimum of at least two groups of nuclear proteins, based upon solubility or lack of it in dilute mineral acid. The acid-soluble proteins, or histones, were also found to contain at least two fractions (77). End-group analysis (82) suggested the presence of 5 to 12 different histones. Studies employing starch gel electrophoresis (83, 84), indicated that 16 to 22 distinct zones were present in samples of nuclear proteins. By chromatography, acid-soluble nuclear proteins (85, 86) from calf thymus have been separated into three to six protein groups. Recent studies on the chromatography of nuclear proteins have combined determination of the distribution of radioactivity in nuclear proteins following injection of labeled amino acids into animals, and determination of the distribution of the protein (61). The Walker and Jensen transplantable rat tumors were found to contain at least eight protein fractions in the acid extracts of purified nuclear preparations while other tissues contained 6 to 10 fractions in similar extracts.

Resistance to Chemotherapeutic Agents

One of the fundamental properties of neoplastic cells that is emerging from studies on chemotherapy is the remarkable ability to adapt to the presence of drugs which are initially potent inhibitors of their metabolism (63). As indicated by Welch (64), a variety of mechanisms may be responsible for the development of "resistance" including altered penetration of the drug into the cells, increased destruction of the drug, increased formation of metabolic antagonists to the drug, increased concentration of the enzyme affected by the drug, decreased requirement for the reaction opposed by the drug, development of an alternative pathway by-passing the metabolite, and formation of enzyme with changed, that is, decreased affinity for the drug (65). Sartorelli & LePage (66) obtained evidence for mutation of neoplastic cells tbat were susceptible to inhibition by azaserine to a strain that was no longer sensitive. The change was stable, irreversible, and heritable, and cross-resistance was found for DON (6-diazo-5-oxo-L-norleucine) and N-methylformamide. The biochemical changes accompanying these developments included an increased ability of tbe resistant strain to synthesize purines *de novo* and an increased ability to utilize preformed purines for the synthesis of nucleic acids. A comparison of cells resistant and sensitive to 5-fluorouracil (67) showed tbat resistant cells were essentially devoid of uridine and deoxyuridine phosphorylases. In addition, there was a marked decrease in tbe concentration of the enzyme, uridine kinase. These experiments indicated tbat the inability of the tumor to be inhibited by fluorouracil might be attributable to the inability of the tumor to activate the fluorouracil by conversion of the drug to the active nucleotide. If the nucleotide were not formed, there would be no inhibition of the reactions leading to the formation of thymine and hence DNA formation would not be inhibited.

Davidson (68) bas demonstrated that the development of resistance to 6-mercaptopurine in a subline of L1210 leukemia was accompanied by a lesser utilization of hypoxanthine. There was very limited conversion of 6-MP or hypoxanthine to ribotides. Davidson reasoned tbat the lack of utilization of hypoxanthine resulted from a competition between 6-MP and hypoxanthine, with the result that insufficient 6-MP ribotide is formed to induce the critical block for purine ribotide formation. The possibility that accumulation of other ribotides might suppress the action of 6-MP was studied by Hakala & Nichol (69), who fonnd that the free base or the ribosides and ribotides of adenine and hypoxanthine prevented the inhibitory effects of 6-MP on the growth of Hela cells. Tbere is much less known about the resistance of tumors tn amethnpterin (70). Permeability changes, changes in enzyme affinities, and other possibilities are now being explored. Many investigators are becoming aware of the enormous practical importance of the subject of the biochemical mechanisms underlying the mutability of the neoplastic cells. If this problem could be solved, there is little doubt that existing antitumor agents could be far mnre effective in therapy.

NUCLEAR PROTEINS

Recent reports (61, 71, 72, 73) have indicated a rapid rate of biosynthesis of nuclear proteins in neoplastic tissues. Comparative studies on a variety of neoplastic tissues, growing non-tumor tissues and non-growing, non-tumor tissues have suggested that the nuclear proteins biosynthesized in the neoplastic cells may be different entities from those synthesized in other tissues (61). These findings have been made possible by the use of radioactive techniques and by the rapid development of refined methods for the isolation and purification of nuclear proteins (74, 75) which have been based upon techniques for fractionation of other proteins (76).

The isolation, identification, and determination of function of the nuclear proteins of neoplastic cells are part of the general problem related to corresponding studies on these proteins in non-tumor cells (77). Perhaps the greatest need confronting biochemists working in this area is the isolation of these proteins in highly purified form. The first problem of initially isolating nuclei in a highly purified form has now been largely solved (1, 2). The studies of Butler's group (78, 79) have established the importance of adequate use of modern methods for characterization of the nuclear proteins, such as sedimentation behavior, solubility, amino acid content, and end-group analysis (80, 81).

Number of nuclear proteins.—Early reports established the presence of a minimum of at least two groups of nuclear proteins, based upon solubility or lack of it in dilute mineral acid. The acid-soluble proteins, or histones, were also found to contain at least two fractions (77). End-group analysis (82) suggested the presence of 5 to 12 different histones. Studies employing starch gel electrophoresis (83, 84), indicated that 16 to 22 distinct zones were present in samples of nuclear proteins. By chromatography, acid-soluble nuclear proteins (85, 86) from calf thymus have been separated into three to six protein groups. Recent studies on the chromatography of nuclear proteins have combined determination of the distribution of radioactivity in nuclear proteins following injection of labeled amino acids into animals, and determination of the distribution of the protein (61). The Walker and Jensen transplantable rat tumors were found to contain at least eight protein fractions in the acid extracts of purified nuclear preparations while other tissues contained 6 to 10 fractions in similar extracts.

RP2-L. One of these fractions, coded as RP2-L (radioactive peak 2, with L-lysine-U-C^{14} as the precursor) has thus far been found in the Walker, Flexner-Jobling and Jensen tumors of the rat, the Morris hepatoma of the rat, the Ehrlich ascites tumor, sarcoma 180 of the mouse, and a human malignant melanoma. This peak has not been found in any of the non-tumor tissues studied, whether they were growing tissues such as regenerating liver or embryonic tissues, or non-growing tissues including spleen, thymus, and small intestine (61). Efforts are presently in progress to characterize the proteins in this radioactive peak and a product has now been obtained which

Resistance to Chemotherapeutic Agents

One of the fundamental properties of neoplastic cells that is emerging from studies on chemotherapy is the remarkable ability to adapt to the presence of drugs which are initially potent inhibitors of their metabolism (63). As indicated by Welch (64), a variety of mechanisms may be responsible for the development of "resistance" including altered penetration of the drug into the cells, increased destruction of the drug, increased formation of metabolic antagonists to the drug, increased concentration of the enzyme affected by the drug, decreased requirement for the reaction opposed by the drug, development of an alternative pathway by-passing the metabolite, and formation of enzyme with changed, that is, decreased affinity for the drug (65). Sartorelli & LePage (66) obtained evidence for mutation of neoplastic cells that were susceptible to inhibition by azaserine to a strain that was no longer sensitive. The change was stable, irreversible, and heritable, and cross-resistance was found for DON (6-diazo-5-oxo-L-norleucine) and N-methylformamide. The biochemical changes accompanying these developments included an increased ability of the resistant strain to synthesize purines *de novo* and an increased ability to utilize preformed purines for the synthesis of nucleic acids. A comparison of cells resistant and sensitive to 5-fluorouracil (67) showed that resistant cells were essentially devoid of uridine and deoxyuridine phosphorylases. In addition, there was a marked decrease in the concentration of the enzyme, uridine kinase. These experiments indicated that the inability of the tumor to be inhibited by fluorouracil might be attributable to the inability of the tumor to activate the fluorouracil by conversion of the drug to the active nucleotide. If the nucleotide were not formed, there would be no inhibition of the reactions leading to the formation of thymine and hence DNA formation would not be inhibited.

Davidson (68) has demonstrated that the development of resistance to 6-mercaptopurine in a subline of L1210 leukemia was accompanied by a lesser utilization of hypoxanthine. There was very limited conversion of 6-MP or hypoxanthine to ribotides. Davidson reasoned that the lack of utilization of hypoxanthine resulted from a competition between 6-MP and hypoxanthine, with the result that insufficient 6-MP ribotide is formed to induce the critical block for purine ribotide formation. The possibility that accumulation of other ribotides might suppress the action of 6-MP was studied by Hakala & Nichol (69), who found that the free base or the ribosides and ribotides of adenine and hypoxanthine prevented the inhibitory effects of 6-MP on the growth of Hela cells. There is much less known about the resistance of tumors to amethopterin (70). Permeability changes, changes in enzyme affinities, and other possibilities are now being explored. Many investigators are becoming aware of the enormous practical importance of the subject of the biochemical mechanisms underlying the mutability of the neoplastic cells. If this problem could be solved, there is little doubt that existing antitumor agents could be far more effective in therapy.

lytic rates of tumors have been related to blood supply (93), environmental conditions affecting metabolism of neoplastic cells (94), and the evidence for similarities in glycolytic pathways of neoplastic and other tissues (95, 96). As indicated by Henderson & LePage (92), there does not seem to be particular differentiation between tumors and other tissues with regard to the vitamins, if consideration is taken of the similarities of biochemical reactions in tumors and other tissues for which the vitamins are required. The metabolism of lipids in tumors has not been found to be different from that of other tissues (97), although for a number of tumors *in vivo* activation of acetate and fluoroacetate was quite limited (98, 99, 100). A variety of tumors have been found to exhibit differences in regard to rates of lipogenesis (101, 102).

Amino acids.—As indicated by Greenstein (103), there are many enzymes of amino acid metabolism which are present in low concentration in neoplastic cells. Auerbach & Waisman (104) have studied the concentration of a number of enzymes involved in catabolism of amino acids in the Novikoff hepatoma and have not been able to demonstrate the presence of tryptophan peroxidase-oxidase, tyrosine transaminase, phenylalanine hydroxylase, threonine dehydrase, serine dehydrase, cysteine desulfhydrase, histidase and *p*-hydroxyphenylpyruvic oxidase. The absence of these enzymes is part of the evidence which indicates that neoplastic tissues tend to streamline to synthetic reactions rather than degradative reactions. Interestingly, Auerbach & Waisman (104) found a fourfold increase in the concentration of aspartic transcarbamylase in the tumor as compared with the concentration in adjacent liver. Since this reaction is on the pathway of pyrimidine synthesis, it may represent a specialization in the same direction as indicated above. A number of tumor cell lines in tissue culture markedly reduce the glutamine content of synthetic media (105). Further interest in the metabolism of asparagine in cell lines has emerged from the findings of McCoy *et al.* (106) that parent cells of Jensen cell lines required asparagine for growth, but the variant strains grew readily in the absence of exogenous asparagine. Christensen *et al.* (107, 108) have suggested that concentration of amino acids by neoplastic tissues exceeds that of other tissues but the mechanisms involved have not been fully defined.

The remarkable ability of tumors to compete successfully for amino acids with the other tissues of the host was exemplified by evidence for rapid growth of neoplasms in animals fed on protein-free diets (109), as well as from studies indicating that carcass nitrogen was used to supply nitrogen to the tumors (110). Evidence that the pathway leading to protein synthesis was not readily reversible in tumors as compared with other tissues, was found in the studies of LePage *et al.* (111) who noted continued rapid growth and uptake of labeled amino acid in tumors while other tissues of the animals bearing tumors suffered marked losses of weight and of protein.

Utilization of plasma proteins.—Although decreased loss of cellular proteins by catabolism of proteins in tumors could explain the findings of LePage

is 92 per cent pure by end-group analysis, that is, 92 per cent of the total N-terminal amino acid is alanine. Amino acid analysis of this protein has revealed that the concentrations of glutamic acid, alanine, leucine, and lysine were high in this protein. A relatively large amount of the total radioactivity, 33 to 45 per cent of the total isotope in acid-soluble nuclear proteins of tumors was found in this radioactive fraction (61).

Metabolism of nuclear proteins.—Evidence for the extensive incorporation of amino acids into nuclear proteins by neoplastic tissues was obtained both *in vivo* and *in vitro* with a variety of amino acids as radioactive precursors (73, 87, 88). Following *in vitro* incubation of the slices or whole cells in media containing balanced salt solutions and radioactive precursors, it was found that the specific activity of nuclear proteins, particularly the acid-extractable nuclear proteins, was equal to or greater than that of cytoplasmic proteins in the neoplastic cells (73, 88, 89). In the non-tumor tissues, the specific activities of the nuclear proteins were uniformly lower than those of the cytoplasmic proteins. The results of experiments *in vivo* with 17 radioactive amino acids as precursors (72, 90), led to the conclusion that an important part of the utilization of amino acids by transplantable rat tumors is the biosynthesis of nuclear proteins, particularly the histones. In the non-tumor tissues studied, biosynthesis of nuclear proteins was evidently a minor cellular function by comparison with other pathways for utilization of the amino acids.

Functions of the nuclear proteins.—Much of our present knowledge of the characteristics of histones or the acid-soluble nuclear proteins results from their ready solubility in a variety of media which permit study of their physical characteristics as well as attempts at their fractionation. However, recent studies (91) have indicated that an important site of attack of mustards and other antitumor agents is on the systems involved in the formation of the acid-insoluble nuclear proteins. In an effort to develop a systematic approach to the study of these proteins, their solubility and chromatographic behavior on diethylaminoethanol cellulose columns was investigated. Present evidence suggests that these proteins, like the acid-soluble nuclear proteins, are a complex mixture of different entities. A variety of concepts have been proposed for their function (77), including the possibilities that they may adhere to the nucleic acids in such a way as to suppress genetic activities, that they may form some sort of combination which enhances the number of genetic potentialities in the chromosomes, or that they may provide a matrix on which the nucleic acids may be joined in the chromosomal sequences. None of these ideas is sufficiently supported by data to permit an adequate opinion as to its validity.

Nutrition of Tumors

Carbohydrates and lipids.—The subject of nutrition of neoplastic cells has recently been most excellently reviewed by Henderson & LePage (92). Interest in carbohydrate metabolism of tumors has decreased since the glyco-

from this laboratory on the fate of radioactive glucose in tumor-bearing rats (127) have demonstrated that in both tumor and non-tumor tissue there was a rapid transfer of the isotope of the radioactive glucose to lactate. The rate at which labeled lactate was formed was greater in brain, heart, and kidney than in the tumor. These findings render unlikely the possibility that a glycolytic inhibitor would be specific for individual reactions of the tumor without interference with other tissues (128, 129).

SPECIAL SUBSTANCES

Some of the special proteins synthesized by neoplastic tissues have received attention in recent investigations. Putnam *et al.* (130) found that different proteins of the Bence Jones group contain different N-terminal amino acids, including leucine, aspartic acid, glutamic acid, or mixtures of aspartic and glutamic acids. Further studies on the terminal amino acids of Bence Jones proteins with aspartic acid as the N-terminal amino acids have been made by Caputo (131). Evidence was obtained that the C-terminal side of the molecule consisted of the sequence leu-ala-glu-val from studies on amino acid liberation with carboxypeptidase.

The antigens of neoplastic tissues have been extensively investigated (132) and Zilber (133) suggested that neoplastic tissues contain antigens which are not present in non-tumor tissues. This fundamental finding, if substantiated, may be related to the presence of nuclear proteins in neoplastic cells which are not found in other tissues (133). Conflicting reports with regard to this point of view (134, 135) have emerged from studies on human and animal tumors.

Mucoproteins.—Fukuda (136) attempted to isolate cancer-specific antigens from the urine of cancer patients and reported that urine from cancer patients contained small quantities of cancer-specific components, as determined by *in vitro* ring precipitin tests, Ouchterlony gel-diffusion, and by immunoelectrophoretic procedures. The responsible components were found to be urinary mucoproteins. Wada *et al.* (137) suggested that physiologically mucoproteins are formed by liver, but in patients with neoplastic disease the tumors were the source of the increased mucoprotein which is found in the blood. Evidence for the production of excess mucoproteins by the Rous sarcoma was obtained by comparison of arterial and venous levels of mucoproteins (138). The close association between toxohormone activity and the mucoproteins of urine and serum of patients with cancer was indicated by the studies of Takahashi (139), who suggested that the toxic substance might be liberated from neoplastic tissues in combination with serum mucoproteins. Winzler *et al.* reported increased concentrations of mucoproteins in plasma of patients with neoplastic disease and suggested that these proteins were α-1 globulins (140).

Toxohormone.—The liver catalase activity of tumor-bearing rats was reported long ago to be one-tenth that of livers of normal rats (103). Injection

et al. (111), the actual uptake of radioactive amino acids by neoplastic cells has been found to be low *in vivo* by comparison with non-tumor tissues (112, 113). A number of studies have now indicated that the uptake of labeled plasma proteins by neoplastic cells exceeds that of other tissues (114 to 117). Studies from this laboratory have indicated that the plasma protein primarily involved in the selective uptake by neoplastic cells is plasma albumin. The isotope from radioactive albumin was incorporated into proteins of the Walker tumor at rates several-fold that of the incorporation into other tissues *in vivo*.

The most notable differences between the uptake of the isotope of labeled albumin and that of the free amino acids are in the differences in the intracellular distribution of the isotope. The isotope of radioactive amino acids is more concentrated in the nuclear proteins and the proteins of the cytoplasmic sap of neoplastic cells. The specific activity of the nuclear proteins was equal to or greater than that of cytoplasmic proteins, particularly those of the mitochondria, when labeled leucine or lysine was employed as the tracer amino acid. When albumin, prelabeled with radioactive leucine or lysine, was employed as a tracer, the specific activity of the proteins of the cytoplasmic sap was twice that of the proteins of other cytoplasmic fractions. The mitochondrial proteins, in turn, were more radioactive than the nuclear proteins.

It would hardly seem possible that the albumin was degraded to the amino acids before it was taken up by the tumor, although the possibility of its partial hydrolysis to peptides has not been ruled out and is at present under study. The explanation for the results with albumin would seem to be best related at this point to pinocytosis (118). Pinocytosis may also account for recently reported evidence for penetration of neoplastic cells by such large molecules as DNA. Selective poisoning of pinocytosis as a nutritional pathway might diminish the growth potential of neoplastic cells without seriously affecting the growth potential of other cells which rely on a more conventional diffusion mechanism for nutrition.

Glycolysis.—More than thirty years ago, Warburg (119) reported that tumors exhibited high rates of both anaerobic and aerobic glycolysis *in vitro*. These findings were interpreted by him (120) as being a characteristic biochemical lesion of neoplasia which resulted from damaged respiration of tumor cells. Exceptions to this theory were soon found in that many tissues, in addition to tumors, have been reported to have high rates of aerobic glycolysis *in vitro* (121). Differences have not beeo found between tumor and non-tumor tissues with regard to content of enzymes of the citric acid cycle (122, 123, 124). Isotope data from this laboratory have indicated that the Krebs cycle in tumors *in vivo* functions to convert glutamate (125) and succinate (126) to lactate. The *in vivo* inactivity of tumors in the oxidation of acetate-1-C^{14} (99, 100), aod in formation of citrate in the fluoroacetate-treated animal (98), may be explaioed on the basis of the low oxygen tension, reduced intratumor pH, and lack of essential substrates (94). Recent data

cause of toxic effects, there was a suppression of marrow function. With the growth of information on such important factors for marrow function as erythropoietin, it is hoped that some of the special effects of neoplastic cells will be more clearly worked out.

Carcinogenesis

Excellent reviews on the present state of information on biochemical mechanisms of induction of oeoplastic disease have recently appeared (150, 151). In addition, there have been many recent reports on the role of viruses in the induction of human neoplasia (152, 153). Although thousands of agents have been shown to have the capacity to induce neoplastic disease (154), thus far, there is a paucity of information on the intimate mechanisms involved. A most challenging observation made by Miller & Miller was that a number of azo dyes combined with the proteins of the liver (151). Heidelberger (155) has provided evidence that the binding of the carcinogens to proteins is primarily by covalent bonds. Thus far, the particular proteins affected by the carcinogenic agents have not been well defined. Since the carcinogens have not yet been shown to interact with nucleic acids which are generally believed to be the genetic transmitting agents, the relationship between protein binding and a genetically transmitted characteristic such as neoplasia needs to be defined. Since the most extensively investigated mechanisms of carcinogenesis are those having to do with chemical carcinogens, it follows that the mechanism of action of other carcinogens will need extensive study before definitive conclusions regarding mechanism can be reached. As Griffin has stated (150):

> It would appear that the formation of tumor cells probably depends upon irreversible changes in the deoxyribonucleoproteins of the cell nucleus. The process occurs gradually as the newly formed cells are exposed to metabolic stress caused by the administration of carcinogens. Many of the endocrine-induced tumors also appear as a result of the cellular hyperplasia which accompanies hormonal imbalances. These dividing cells are also exposed to a greatly altered environment. Many of the other carcinogens act directly on deoxyribonucleoprotein and nuclear metabolism. . . . With our advancing knowledge of methods of isolation and characterization of macromolecules, we may find the deoxyribonucleoproteins of malignant cells are different from those of normal cells.

Summary

What remains to be done? First, it is necessary to isolate and characterize the constituents of the nucleus in terms of structure and function, that is, the structures and functions of each of the 46 human chromosomes must be established, particularly with reference to the biological activities of the constituent deoxyribonucleic acids (DNA) and the proteins. In addition, the role of the nuclear membrane in the somatic cell must be defined as well as the change in the membrane in mitosis, normal and neoplastic. The types and functions of nuclear ribonucleic acids (RNA) must be ascertained. Nu-

of extracts of tumors resulted in a decrease in the catalase activity of livers of normal rats. Extirpation of the tumors rapidly restored liver catalase while inoculation of a second tumor in those animals from which the original tumor had been removed caused a second decrease in liver catalase activity. A comparison of benign and malignant tumors of the rat (141) revealed only an 8 per cent decrease in liver catalase activity of animals bearing mammary fibroadenoma. When this tumor underwent spontaneous transformation to a fibrosarcoma, a decrease resulted of approximately 50 per cent in liver catalase activity.

The concept of a toxic bormone-like agent, "toxohormone" liberated by the tumor has been extended by the work of Nakahara & Fukuoka (142). They obtained toxohormone by aqueous extraction of tumors followed by precipitation with alcohol. Toxohormone was thermostable, readily soluble in water but not in ether, alcohol precipitable, and active in 100 mg. doses.

Structure of toxohormone.—Acid hydrolysis of toxohormone yielded a number of amino acids (143). A substance with toxohormone-like activity was produced by tumor slices on incubation with arginine, phenylalanine, leucine, and ATP. Slices of normal tissues, however, were not capable of producing this active substance under the same experimental conditions (142). Recently, Yunoki & Griffin (144) have separated crude toxohormone from human tumors into three chromatographic fractions utilizing Amberlite XE-64. These tbree fractions were capable of reducing liver catalase activity *in vivo* in doses of 10 mg., 1 to 5 μg., and 10 μg., respectively. The most active fraction appears to be a dialyzable polypeptide of low molecular weight.

Anemia in Cancer Patients

Among the causative factors for anemia in cancer patients is overutilization by tumors of substrates essential for the function of the marrow (110, 111, 115), althougb other factors such as marrow displacement and chronic bleeding may account for the anemias of leukemia and the anemias attendant upon lesions in hollow viscera. The "unexplained anemia" in neoplastic disease is a clinical concept in which the degree of anemia seems to be greater than would be accounted for by either the size of tbe tumor or by its location. In studies on this subject, Greenfield *et al.* (145, 146, 147) reported that large amounts of iron in red cells localized in transplantable rat tumors. When tbe red cells were lysed, this pheoomenoo did oot occur, providing evidence for entry of whole red cells into tumors and their subsequent destruction within the tumor mass. The slow reutilizatioo of the iron deposited suggested the possibility that part of the anemia results from the inability of the body to return the iron from the tumor mass to the body pool (148).

Hemolysis accounts in part for the development of anemia in patients with lymphomas and leukemia (149). The anemia resulting from bemolysis produced a variable erythropoietic response in the bone marrow. Desforges *et al.* (149) concluded that either because of lack of growth factors or be-

LITERATURE CITED

1. Chauveau, J., Moulé, Y., and Rouillier, C. H., *Exptl. Cell Research*, 11, 217–21 (1956)
2. Busch, H., Starbuck, W. C., and Davis, J. R., *Cancer Research*, 19, 684–87 (1959)
3. Zubay, G., and Doty, P., *J. Mol. Biol.*, 1, 1–20 (1959)
4. Hamilton, L. D., Barclay, R. K., Wilkins, M. H. F., Grown, G. L., Wilson, H. R., Marvin, D. A., Ephrussi-Taylor, H., and Simmons, N. S., *J. Biophys. Biochem. Cytol.*, 5, 397–404 (1959)
5. Watson, J. D., and Crick, F. H. C., *Nature*, 171, 737 (1953)
6. Watson, J. D., and Crick, F. H. C., *Nature*, 171, 964 (1953)
7. Sinsheimer, R. L., *J. Mol. Biol.*, 1, 43–53 (1959)
8. Vendrely, R., Vendrely, C., and Shadron, C., *Exptl. Cell. Research*, 15, 222–29 (1958)
9. Kleinschmidt, W. J., *Cancer Research*, 19, 966–69 (1959)
10. Kit, S., *Arch. Biochem. Biophys.*, 87, 318–29 (1960)
11. Kit, S., *Arch. Biochem. Biophys.*, 87, 330–36 (1960)
12. Kit, S., *Arch. Biochem. Biophys.*, 88, 1–9 (1960)
13. Sirlin, J. L., and Knight, G. R., *Exptl. Cell. Research*, 19, 210–19 (1960)
14. Bendich, A., Pahl, H. B., Korngold, G. C., Rosenkranz, H. S., and Fresco, J. R., *J. Am. Chem. Soc.*, 80, 3949–56 (1958)
15. Kondo, N., and Osawa, S., *Nature*, 183, 1602 (1959)
16. Petrakis, N. L., Bostick, W. L., and Siegel, B. V., *J. Natl. Cancer Soc.*, 22, 551–54 (1959)
17. Giordano, A., Pecchiai, L., and Rilke, F., *Riv. isotochim. norm. e patol.*, 4, 275–320 (1958)
18. Scarlato, G., and Muller, W., *Acta Histochem.*, 6, 240–53 (1959)
19. Josse, J., and Kornberg, A., *Federation Proc.*, 19, 305 (1960)
20. Burton, K., *Biochem. J.*, 74, 35P (1960)
21. Razzell, W. E., and Khorana, H. G., *J. Biol. Chem.*, 234, 2114–17 (1959)
22. Kit, S., and Griffin, A. C., *Cancer Research*, 18, 621–56 (1958)
23. Smith, C. L., Newton, A. A., and Wildy, P., *Nature*, 184, 107–8 (1959)
24. Smellie, R. M. S., Gray, E. D., Keir, H. M., Richards, J., Bell, D., and Davidson, J. N., *Biochim. et Biophys. Acta*, 37, 243–50 (1960)
25. Bessman, M. J., Lehman, I. R., Simms, E. S., and Kornberg, A., *J. Biol. Chem.*, 233, 171–77 (1958)
26. Bender, M. A., and Mettler, L. E., *Science*, 128, 186–90 (1958)
27. Hsu, T. C., *J. Heredity*, 43, 167–72 (1952)
28. Hsu, T. C., and Pomerat, C. M., *J. Heredity*, 44, 23–29 (1953)
29. Barner, H. D., and Cohen, S. S., *J. Biol. Chem.*, 234, 2987–91 (1959)
30. Flaks, J. G., and Cohen, S. S., *J. Biol. Chem.*, 234, 1501–6 (1959)
31. Flaks, J. G., Lichtenstein, J., and Cohen, S. S., *J. Biol. Chem.*, 234, 1507–11 (1959)
32. Flaks, J. G., and Cohen, S. S., *J. Biol. Chem.*, 234, 2981–86 (1959)
33. Henderson, J. F., and LePage, G. A., *Cancer Research.*, 19, 67–71 (1959)
34. Hurlbert, R. B., and Kammen, H. O., *J. Biol. Chem.*, 235, 443–49 (1960)
35. Rutman, R. J., Cantarow, A., and Paschkis, K. E., *Cancer Research*, 14, 119–23 (1954)
36. Heidelberger, C., Leibman, K. C., Harbers, E., and Bhargava, P. M., *Cancer Research*, 17, 399–404 (1957)
37. Yates, R. A., and Pardee, A. B., *J. Biol. Chem.*, 227, 677–92 (1957)
38. Wyngaarden, J. B., and Ashton, D. M., *Nature*, 183, 747–48 (1959)
39. Bresnick, E., *Proc. Am. Assoc. Cancer Research*, 3, 98 (1960)
40. Potter, V. R., *Univ. Mich. Med. Bull.*, 23, 401–12 (1957)
41. Peters, T., Jr., *J. Histochem. and Cytochem.*, 7, 224–34 (1959)
42. Keller, E. B., Zamecnik, P. C., and Loftfield, R. B., *J. Histochem. and Cytochem.*, 2, 378–86 (1954)
43. Campbell, P. N., *Advances in Cancer Research*, 5, 97–155 (1958)
44. Rendi, R., and Campbell, P. N., *Biochem. J.*, 72, 435–41 (1959)
45. Korner, A., *Biochem. et Biophys. Acta*, 35, 554–55, (1959)
46. Cohn, P., *Biochim. et Biophys. Acta*, 33, 284–85 (1959)
47. Berg, P., *J. Biol. Chem.*, 233, 601–7 (1958)
48. Brachet, J., *Nature*, 186, 194 (1960)
49. Sirlin, J. L., *Nature*, 186, 275 (1960)
50. Holley, R. W., and Doctor, B. P.,

clear events must be understood from the time of separation of centrosomes through the formation of spindles and the separation of the chromosomes. In addition, the role of the nucleolus as well as the nuclear sap must be established, particularly in terms of nuclear-cytoplasmic relationships. Each of these studies will be a long-term effort and the definition of the differences between the neoplastic and other cells will be a demanding task. However, in 1961, one can take much satisfaction from the fact that the task is under way and fundamental information is being obtained at a progressively more rapid pace.

100. Busch, H., and Baltrush, H. A., *Cancer Research*, 14, 448–55 (1954)
101. Begg, R. W., and Trew, J. A., *Federation Proc.*, 16, 152 (1957)
102. Jablonski, J. R., and Olson, R. E., *Proc. Am. Assoc. Cancer Research*, 2, 26 (1955)
103. Greenstein, J., *Biochemistry of Cancer* (Academic Press, New York, N. Y., 1954)
104. Auerbach, V. H., and Waisman, H. A., *Cancer Research*, 18, 536–42 (1958)
105. Roberts, E., and Borges, P. R. F., *Cancer Research*, 15, 697–99 (1955)
106. McCoy, T. A., Maxwell, M. D., Irvine, E., and Sartorelli, A. C., *Proc. Soc. Exptl. Biol. Med.*, 100, 862–65 (1959)
107. *Christensen, H. N., and Riggs, T. R., J. Biol. Chem.*, 194, 57–68 (1952)
108. Christensen, H. N., Rothwell, J. T., Sears, R. A., and Streicher, J. A., *J. Biol. Chem.*, 175, 101–5 (1948)
109. White, F. R., and Belkin, M., *J. Natl. Cancer Inst.*, 5, 261–64 (1945)
110. Sherman, C. D., Jr., Morton, J. J., and Mider, G. B., *Cancer Research*, 10, 374–78 (1950)
111. LePage, G. A., Potter, V. R., Busch, H., Heidelberger, C., and Hurlbert, R. B., *Cancer Research*, 12, 153–57 (1952)
112. Busch, H., and Greene, H. S. N., *Yale J. Biol. and Med.*, 27, 339–49 (1955)
113. Zamecnik, P. C., Loftfield, R. B., Stephenson, M. L., and Steele, J. M., *Cancer Research*, 11, 592–602 (1951)
114. Babson, A. L., and Winnick, T., *Cancer Research*, 14, 606–11 (1954)
115. Busch, H., Simbonis, S., Anderson, D. C., and Greene, H. S. N., *Yale J. Biol. and Med.*, 29, 105–16 (1956)
116. Campbell, P. N., Jones, H. E. H., and Stone, N. E., *Nature*, 177, 139 (1956)
117. Kent, H. N., and Gey, G. O., *Proc. Soc. Exptl. Biol. Med.*, 94, 205–9 (1957)
118. Lewis, W. H., *Bull. Johns Hopkins Hosp.*, 49, 17–28 (1931)
119. Warburg, O., *Metabolism of Tumors* (Richard R. Smith, Inc., Publishers, New York, 1931)
120. Warburg, O., *Science*, 123, 309–14 (1956)
121. Dickens, F., and Weil-Malherbe, H., *Biochem. J.*, 35, 7–15 (1941)
122. Weinhouse, S., *Cancer Research*, 11, 571–84 (1951)
123. Jedeikin, L. A., and Weinhouse, S., *J. Biol. Chem.*, 213, 271–80 (1955)
124. Barban, S., and Schulze, H. O., *J. Biol. Chem.*, 222, 665–70 (1956)
125. Nyhan, W. L., and Busch, H., *Cancer Research*, 18, 385–93 (1958)
126. Nyhan, W. L., and Busch, H., *Cancer Research*, 18, 1203–8 (1958)
127. Busch, H., Fujiwara, E., and Keer, L. M., *Cancer Research*, 20, 50–57 (1960)
128. Busch, H., *Cancer Research*, 15, 365–74 (1955)
129. Busch, H., Amer, S. M., and Davis, J. R., *J. Am. Pharm. Assoc. Sci. Ed.*, 49, 16–17 (1960)
130. Putnam, F. W., *Physiol. Revs.*, 37, 512–38 (1957)
131. Caputo, A., *Clin. Chim. Acta*, 4, 545–48 (1959)
132. Southam, C., *Cancer Research*, 20, 271–92 (1960)
133. Zilber, L. A., *Acta Unio. Intern. Contra Cancrum*, 15, 933–39 (1959)
134. Kosyakov, P. N., and Korosteleva, V. S., *Bull. Exptl. Biol. Med.*, 47, 226–30 (1959) (Russian Trans.)
135. Airapet'yan, G. P., *Bull. Exptl. Biol. Med.*, 48, 870–74 (1959) (Russian Trans.)
136. Fukuda, M., *Sapporo Igaku Zasshi*, 14, 250–73 (1958)
137. Wada, T., Ohara, H., Sasaki, T., Nakajima, J., and Yachi, A., *Gann*, 48, 305–14 (1957)
138. Sasaki, T., *Sapporo Igaku Zasshi*, 11, 362–72 (1957)
139. Takahashi, R., *Sapporo Igaku Zasshi*, 11, 373–84 (1957)
140. Winzler, R. J., and Smyth, I. M., *J. Clin. Invest.*, 27, 617–19 (1948)
141. Begg, R. W., Dickenson, T. E., and Millar, J., *Can. J. Med. Sci.*, 31, 315–19 (1953)
142. Nakahara, W., and Fukuoka, F., *Advances in Cancer Research*, 5, 157–77 (1958)
143. Adams, O. H., *Brit. J. Cancer*, 4, 183–95 (1950)
144. Yunoki, K., and Griffin, A. C., *Cancer Research*, 20, 533–40 (1960)
145. Greenfield, R. E., Godfrey, J. E., and Price, V. E., *J. Natl. Cancer Inst.*, 21, 641–56 (1958)
146. Greenfield, R. E., Sterling, W. R., and Price, V. E., *J. Natl. Cancer Inst.*, 21, 1099–1107 (1958)
147. Greenfield, R. E., Sterling, W. R., and Price, V. E., *J. Natl. Cancer Inst.*, 24, 87–96 (1960)
148. Price, V. E., Greenfield, R. E., Sterling W. R., and MacCardle, R. C., *J.*

Federation Proc., 19, 348 (1960)

51. Holley, R. W., and Merrill, S. H., *J. Am. Chem. Soc.*, 81, 753 (1959)
52. Goldthwait, D. A., *J. Biol. Chem.*, 234, 3245–50 (1959)
53. Kirby, K. S., *Biochim. J.*, 64, 405–8 (1956)
54. Sibitani, A., Yamana, K., Mirima, K., and Takahashi, T., *Nature*, 186, 215–17 (1960)
55. Chargaff, E., Magasanik, B., Vischer, E., Green, C., Doniger, R., and Elson, D., *J. Biol. Chem.*, 186, 51–67 (1950)
56. deLamirande, G., Allard, C., and Cantero, A., *Cancer Research*, 15, 329–32 (1955)
57. Caspersson, T., and Santesson, L., *Acta Radiol. Suppl.*, 46 (1942–43)
58. Stenram, U., *Acta Pathol. Microbiol. Scand.*, 44, 239 (1958)
59. Love, R., and Liles, R. H., *J. Histochem. and Cytochem.*, 7, 164–81 (1959)
60. Swift, H., *Symposium on Mol. Biol., Univ. Chicago*, 266–303 (1959)
61. Davis, J. R., and Busch, H., *Cancer Research*, 19, 1157–66 (1959)
62. Bijvoet, P., and Busch, H., *Federation Proc.*, 19, 318 (1960)
63. Skipper, H. E., and Bennett, L. L., Jr., *Ann. Rev. Biochem.*, 27, 137–66 (1959)
64. Welch, A., *Cancer Research*, 19, 359–71 (1959)
65. Davis, B. D., and Maas, W. K., *Proc. Natl. Acad. Sci., U.S.*, 38, 775–85 (1952)
66. Sartorelli, A. C., and LePage, G. A., *Cancer Research*, 18, 457–63 (1958)
67. Reichard, P., Skold, O., and Klein, G., *Nature*, 183, 939–41 (1959)
68. Davidson, J. D., *Cancer Research*, 20, 225–32 (1960)
69. Hakala, M. T., and Nichol, C. A., *J. Biol. Chem.*, 234, 3224–28 (1959)
70. Aranow, L., *J. Pharmacol., Exptl. Therap.*, 127, 116–21 (1959)
71. Busch, H., Davis, J. R., and Anderson, D. C., *Cancer Research*, 18, 916–26 (1958)
72. Busch, H., Davis, J. R., Honig, G. R., Anderson, D. C., Nair, P. V., and Nyhan, W. L., *Cancer Research*, 19, 1030–39 (1959)
73. Starbuck, W. C., and Busch, H., *Cancer Research*, 20, 819–96 (1960)
74. Davison, P. F., *Biochem. J.*, 66, 703–7 (1957)
75. Davison, P. F., *Biochem. J.*, 66, 708–12 (1957)
76. Peterson, E. A., and Sober, H. A., *J. Am. Chem. Soc.*, 78, 751–55 (1956)
77. Busch, H., and Davis, J. R., *Cancer Research*, 19, 1241–56 (1958)
78. Davison, P. F., James, D. W. F., Shooter, K. V., and Butler, J. A. V., *Biochim. et Biophys. Acta*, 15, 415–24 (1954)
79. Davison, P. F., and Butler, J. A. V., *Biochim. et Biophys. Acta*, 15, 439–40 (1954)
80. Phillips, D. M. P., *Biochim. J.*, 68, 35–40 (1958)
81. Phillips, D. M. P., and Johns, E. W., *Biochem. J.*, 72, 538–44 (1959)
82. Luck, J. M., Rasmussen, P. S., Satake, K., and Tsvetikov, A. N., *J. Biol. Chem.*, 233, 1407–14 (1958)
83. Neelin, J. M., and Connell, G. E., *Biochim. et Biophys. Acta*, 31, 539–41 (1959)
84. Neelin, J. M., and Neelin, E. M., *Can. J. Biochem. and Physiol.*, 38, 355–63 (1960)
85. Crampton, C. F., Stein, W. H., and Moore, S., *J. Biol. Chem.*, 225, 363–86 (1957)
86. Neelin, J. M., and Butler, G. C., *Can. J. Biochem. and Physiol.*, 37, 843–59 (1959)
87. Davis, J. R., and Busch, H., *Cancer Research*, 18, 718–24 (1958)
88. Busch, H., Davis, J. R., and Anderson, D. C., *Acta Unio. Intern. Contra Cancrum*, 16, 1125–31 (1960)
89. Campbell, P. N., Greengard, O., and Jones, H. E. H., *Exptl. Cell Research*, 12, 689–92 (1957)
90. Rotherham, J., Irvin, J. L., Irvin, E. M., and Holbrook, D. J., Jr., *Proc. Soc. Exptl. Biol. Med.*, 96, 21–24 (1957)
91. Busch, H., Amer, S. M., and Nyhan, W. L., *J. Pharmacol. Exptl. Therap.*, 127, 195–99 (1959)
92. Henderson, J. F., and LePage, G. A., *Cancer Research*, 19, 887–902 (1959)
93. Urbach, F., and Noell, W. K., *Proc. Am. Assoc. Cancer Research*, 2, 257 (1957)
94. Busch, H., Davis, J. R., and Olle, E. W., *Cancer Research*, 17, 711–16 (1957)
95. LePage, G. A., *Cancer Research*, 10, 77–83 (1950)
96. Weinhouse, S., *Advances Cancer Research*, 3, 269–325 (1955)
97. Medes, G., and Weinhouse, S., *Cancer Research*, 18, 352–59 (1958)
98. Potter, V. R., and Busch, H., *Cancer Research*, 10, 353–56 (1950)
99. Busch, H., *Cancer Research*, 13, 789–94 (1953)

NEOPLASTIC DISEASE: TUMOR-VIRUS RELATIONS[1]

By Sarah E. Stewart, Ph.D., M.D.

National Cancer Institute, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare, Bethesda, Maryland

Properties of Viruses

Cellular changes caused by viruses.—The discovery of viruses as disease-producing agents led to many discussions concerning their nature and that of the diseases produced by them. A great deal is known regarding the properties of many of the viruses such as their size, shape, reaction to physical agents, chemical constitution, and the antigenic responses they produce in the infected host. By means of epidemiological studies, much has also been learned of their mode of transmission.

It is now known that viruses replicate within susceptible cells, often producing pathologic effects. Both proliferative and degenerative changes accompanied by inflammatory reactions, generally with infiltration of mononuclear cells, are found in most active virus infections. One of the first degenerative changes commonly observed is a ballooning degeneration of the nucleus or of the whole cell, resulting in its destruction. With certain virus infections it is difficult to determine whether proliferative or degenerative lesions appear first.

In those diseases affecting neurons, such as poliomyelitis and rabies, proliferation has not been observed as these cells are incapable of multiplication. Also in infections, such as those caused by the virus of foot-and-mouth disease, the necrotizing action is so rapid that hyperplasia has not been described. With the oncogenic viruses, hyperplasia of the affected cells is the predominant effect and a necrotizing action may not be observed.

Influence of host age on viral activity.—The effect produced by a given virus will vary depending on the susceptibility of the host as determined by such factors as its immunological state, genetic constitution, hormonal balance, etc., but depends greatly on the age of the host. An oncogenic virus in a young animal may produce a generalized disease instead of the neoplasm it produces in the older animal. This was demonstrated by Duran-Reynals early in his work with the Rous sarcoma virus. He found that the sarcoma virus caused a fatal generalized hemorrhagic lesion when inoculated into chicks and not sarcomas as it does in older fowl (1). He also showed that newborn rabbits inoculated with the Shope fibroma virus developed a fatal inflammatory disease rather than the benign fibromas (2). He concluded that viral infection of the older and generally more resistant host may manifest itself preferentially by cell proliferation rather than by cell destruction. Furthermore, it is known that only newborn or suckling animals are susceptible to infection with certain viruses. The Coxsackie virus is an example of an

[1] The survey of the literature pertaining to this review was concluded in 1960.

Natl. Cancer Inst., 22, 877–85 (1959)
149. Desforges, J. F., Ross, J. D., and Moloney, W. C., *Am. J. Med.*, 28, 69–76 (1960)
150. Griffin, A. C., *Biochemistry of Cancer Induction in Cancer*, 123–60 (R. W. Raven, Ed., Butterworths Sci. Publs., London, Engl., 1957)
151. Miller, E. C., and Miller, J. A., *Ann. Rev. Biochem.*, 28, 291–320 (1959)
152. Dmochowski, L., "The Part Played by Viruses in the Origin of Tumors," in *Cancer*, 214–305 (Raven, R. W., Ed., Butterworths Sci. Publs., London, Engl., 1957)
153. Grace, J. T., Mirand, E. A., Mount, D. T., and Metzgar, R., *Proc. Am. Assoc. Cancer Research*, 3, 115 (1960)
154. Hueper, W. C., *Occupational Tumors and Allied Diseases* (Charles C Thomas, Publ., Springfield, Ill., 1942)
155. Heidelberger, C., *Acta Unio. Intern. Contra Cancrum*, 15, 107–13 (1959)

with the bacterial genome without producing visible changes. These bacteria which carry the viral genome are known as lysogenic bacteria. Some of them may release phage spontaneously, others only by induction with some external stimulus such as x-ray or ultraviolet light. Still others cannot be made to release bacteriophage. Here we may have an analogy to what occurs with some viruses that become latent in mammalian tissues.

The phage in a lysogenic bacterium is passed on to daughter cells at cell division since the phage genome becomes attached to the bacterial chromosomes. In this stage it is known as "prophage." Alterations in the genetic characters of bacteria are known to be produced by bacteriophage. These can be brought about by two mechanisms, conversion and transduction (13). In conversion, the change is produced by an interacting of the viral genome on the cell. The effect produced by certain tumor viruses on cells resulting in their transformation to malignancy has been likened to the conversion induced in bacteria by bacteriophage. Temin (14) has demonstrated an hereditary change in Rous sarcoma cells produced by a mutant of the Rous virus. When chick fibroblasts in tissue culture were inoculated with "normal" Rous virus they were transformed from elongated into rounded cells that were indistinguishable from epithelial cells. With a mutant of the virus, the infected fibroblasts became transformed to very thin fusiform cells; intermediate forms with other mutants were also observed. In these experiments, the transformed dividing cell passed to its progeny the newly acquired characteristic and produced virus of the mutant type.

In the second mechanism, transduction, the change is brought about when a temperate phage transfers genes from a bacterium in which it has replicated to a newly infected cell, producing changes in it. It becomes a permanent change if the new gene is incorporated in the host chromosome; otherwise, it is known as an abortive transduction.

Theoretical Speculations on the Mode of Action of Polyoma Virus

Polyoma virus, an oncogenic virus which was first demonstrated in cell-free extracts of mouse leukemic tissues by Stewart (15, 16, 17), and was later cultivated in tissue cultures by Stewart, Eddy and their collaborators (18, 19, 20), has many of the properties of the usual types of viruses. For a characterization of the virus, the reader is referred to published work (11, 21 to 26). Although the mechanism whereby polyoma virus produces malignant transformation of cells is not known, similarities in the behavior of this virus with the bacterial viruses exist. Newborn mice and hamsters inoculated with polyoma virus develop within a few days a ballooning degeneration of the nuclei and lysis of many of the infected cells (21). It is in these cells that virus has been demonstrated by electron microscopy (27, 28), and in similarly enlarged cells in mouse embryo cultures by both electron microscopy (29) and by the application of the fluorescent antibody method (30). The cells in which the virus is replicating are destroyed and it is the neighboring cells of the same

infectious virus that produces disease in mice only if they are inoculated during the suckling stage (3). Of the oncogenic viruses, polyoma virus is probably one of the best examples for demonstrating the effect of age on susceptibility. Resistance of mice to tumor induction by the virus increases very rapidly after they reach 24 hr. of age (4).

Virus latency and factors which influence host response.—Latent infections have been demonstrated with both the oncogenic and the usual types of viruses. Infection with the virus of herpes simplex frequently results in recurrent attacks. Individuals who have recovered from this infection become carriers of the virus and many, in spite of antibodies which they have developed against it, have recurrent manifestations of herpetic infection following non-specific stimuli such as fever or emotional upsets. The Bittner milk agent which produces mammary adenocarcinomas in mice will lie dormant in the infected female until it becomes activated by the normal estrogen secretions. Outside stimuli are also known to activate latent oncogenic viruses. Radiation produces leukemia in some strains of mice by activating a latent leukemia virus (5, 6). Cortisone has been reported to produce parotid gland tumors in mice (7), probably by the activation of latent polyoma virus.

Epidemiology.—Certain of the tumor viruses are known to be contagious and their mode of spread is similar to that of other viruses. Burmester and associates (8) demonstrated that natural infection with chicken visceral lymphomatosis virus occurs, and that infection may result from exposure to saliva and feces from infected birds. Contact transmission of Rous sarcoma also has been shown to occur (9). Mosquito transmission of the Shope fibroma and squirrel fibroma virus has been demonstrated (10). Polyoma virus also has been shown to be contagious. Uninoculated mice housed in the same room with but in separate cages from inoculated animals were found to develop antibodies to the virus (11). Latent infections were found in certain mouse colonies; in some instances 80 per cent of the mice had polyoma virus antibodies (12). The mode of spread was shown to be by means of saliva and excreta, principally the urine.

Similarities between animal viruses and bacterial viruses.—As our knowledge of the behavior of viruses increases, more and more similarities are found not only between the non-tumor viruses and those which produce tumors, but also between the animal viruses and bacterial viruses. In order to discuss the possible analogies which may exist between animal viruses and bacteriophage, a review of some of the pertinent findings and definition of terms follows. When a vegetative or virulent phage enters a sensitive bacterial cell it may replicate in the cell, resulting in its lysis. This is brought about by the phage genome which is composed of the viral nucleic acid. It penetrates the bacterial cell and induces the bacterium to reproduce more of the viral nucleic acid. Essentially, this is what also occurs when a virulent animal virus enters a mammalian cell, resulting in its destruction.

In other bacterial cells, the phage genome from a temperate (non-virulent) bacteriophage on entering the bacterial cell may go into a stable relationship

with the bacterial genome without producing visible changes. These bacteria which carry the viral genome are known as lysogenic bacteria. Some of them may release phage spontaneously, others only by induction with some external stimulus such as x-ray or ultraviolet light. Still others cannot be made to release bacteriophage. Here we may have an analogy to what occurs with some viruses that become latent in mammalian tissues.

The phage in a lysogenic bacterium is passed on to daughter cells at cell division since the phage genome becomes attached to the bacterial chromosomes. In this stage it is known as "prophage." Alterations in the genetic characters of bacteria are known to be produced by bacteriophage. These can be brought about by two mechanisms, conversion and transduction (13). In conversion, the change is produced by an interacting of the viral genome on the cell. The effect produced by certain tumor viruses on cells resulting in their transformation to malignancy has been likened to the conversion induced in bacteria by bacteriophage. Temin (14) has demonstrated an hereditary change in Rous sarcoma cells produced by a mutant of the Rous virus. When chick fibroblasts in tissue culture were inoculated with "normal" Rous virus they were transformed from elongated into rounded cells that were indistinguishable from epithelial cells. With a mutant of the virus, the infected fibroblasts became transformed to very thin fusiform cells; intermediate forms with other mutants were also observed. In these experiments, the transformed dividing cell passed to its progeny the newly acquired characteristic and produced virus of the mutant type.

In the second mechanism, transduction, the change is brought about when a temperate phage transfers genes from a bacterium in which it has replicated to a newly infected cell, producing changes in it. It becomes a permanent change if the new gene is incorporated in the host chromosome; otherwise, it is known as an abortive transduction.

Theoretical Speculations on the Mode of Action of Polyoma Virus

Polyoma virus, an oncogenic virus which was first demonstrated in cell-free extracts of mouse leukemic tissues by Stewart (15, 16, 17), and was later cultivated in tissue cultures by Stewart, Eddy and their collaborators (18, 19, 20), has many of the properties of the usual types of viruses. For a characterization of the virus, the reader is referred to published work (11, 21 to 26). Although the mechanism whereby polyoma virus produces malignant transformation of cells is not known, similarities in the behavior of this virus with the bacterial viruses exist. Newborn mice and hamsters inoculated with polyoma virus develop within a few days a ballooning degeneration of the nuclei and lysis of many of the infected cells (21). It is in these cells that virus has been demonstrated by electron microscopy (27, 28), and in similarly enlarged cells in mouse embryo cultures by both electron microscopy (29) and by the application of the fluorescent antibody method (30). The cells in which the virus is replicating are destroyed and it is the neighboring cells of the same

or different type that develop malignant transformation. Leuchtenberger *et al.*, in a correlated cytolngical, histnlogical, and cytochemical study on polyoma virus-infected mice, reported a difference in hnst-cell-virus relationship between tubular epithelium and stroma in kidneys of inoculated mice (31). In this study it was found that after polyoma virus inoculation, infection of epithelial cells, followed by the death of such cells and the liberation of virus particles, always preceded the proliferation nbserved in the stromal cells. Proliferation of the tubular epithelial cells is also knnwn to nccur as was reported earlier (18, 19). The tubular epithelial cell proliferation perhaps is more frequent in the strain C_3H_f/Hen mice and (C_3H_f/Hen$\times$AKR)F_1 hybrids used in the earlier studies than in the Swiss mice used in the Leuchtenberger study. Here we have a very good example of a virus that produces both a necrotizing effect in certain cells and a proliferative effect in others in the same hnst.

Is the effect produced dependent on the host rather than on the virus as pninted out by Duran-Reynals? Prnbably both influence the end result. As already mentinned, the initial response observed in the tissues nf mice and hamsters that received polyoma virus within a few hours after birth is a necrntizing effect. This nccurs within a few days after infection. Epithelial cells, particularly in the kidney tubules, are the target cells fnr virus replicatinn. Very early in infection only the ballooning degeneration of the nucleus is present; in mice three to four mnnths old only an nccasinnal cell thus affected can still be fnund. These are seen most frequently in the kidneys and in thyroid glands undergoing neoplastic changes. In the hamster, the perind of virus replication as evidenced by the enlarged epithelial cells is nf even shorter duration. Needless to say, polyoma virus is readily recoverable at this early stage. What happens tn the host cells that prevents continuus replication of virus and destruction of the cells but instead activates the proliferative effect? It has been shown that polyoma virus is highly antigenic and that animals inoculated with it develop antibodies that can be detected within a short time after infection (11, 21, 32). Antibodies, however, do not prevent the replication of the virus or the malignant transformation of cells if introduced after the virus has entered the cells (11). High serum antibody titers have been demonstrated in mice bearing large polyoma virus-induced tumors with occasional cells showing the ballooning degeneration (11). The change from necrosis to proliferation cannot then be attributed to host resistance produced by specific antibodies. A similar change in response from necrosis to proliferation has been observed in tissue cultures of mouse embryo cells inoculated with polyoma virus. The initial response observed in cultures is the necrotizing effect. As polyoma virus appears to be slow-growing, the tissue culture cytopathogenic changes usually are not observed until seven days after the virus inoculation (24). From 9 to 14 days many of the cells in the culture are destroyed. Recently it has been reported that following cell lysis remaining cells in some instances undergo a proliferative response (33). A marked decrease in the amount of recoverable virus from the proliferative

cultures was observed. A proliferative response has also been reported for hamster embryo tissue cultures inoculated with the virus. In these no cell lysis was observed (34, 35). Only cloning of cells will determine whether virus is produced by the proliferating cells. Both tissue susceptibility and virus virulence may be the determining factors. If certain cells in the host are highly susceptible to viruses, they undergo lysis as a result of their replication. Others not as susceptible react to the virus as bacteria would to a temperate phage. There may also be a difference in the virulence of different virus particles.

The following are given as examples of host resistance that also are not attributable to specific viral antibodies: (*a*) Development of benign tumors. Rabbits inoculated subcutaneously shortly after birth with the supernatant fluid from a polyoma virus mouse embryo culture develop subcutaneous tumors in two to three weeks (36, 37). Histologically these resemble benign fibromas that will grow for a period and then regress [attempts to reactivate the tumors by administering hydrocortisone have failed (38)]. (*b*) Development of resistance with age. Resistance to the oncogenic effects of the virus increases with age. Hamsters over 30 days of age do not develop tumors when inoculated with it. Resistance in mice develops after they reach 24 hr. of age (4).

Tumor induction in the rabbit and regression of the tumors could be explained as resulting from a transient type of transduction in the following manner: when polyoma virus which has been replicating in mouse embryo tissue culture is inoculated into newborn rabbits, the viral genome may transfer genes or proteins from mouse cells to the rabbit cells. The rabbit cells, either because of the transferred genes or because of a tolerance acquired from the injected mouse protein, are now susceptible to transformation by the virus. The viral genome apparently does not become permanently integrated with the rabbit chromosomes. A permanent type of change therefore does not occur, the change being transmitted to daughter cells for a limited number of generations, resulting in tumor regression.

In hamsters the situation is different in that the tumors do not regress, but it is similar in that a mouse virus replicating in mouse cells produces a similar type of transduction; in this case it is a permanent hereditary change.

In recent studies, purified nucleic acid (DNA) obtained from polyoma virus mouse embryo cultures was found to induce tumors when inoculated directly into hamsters (39). Had the DNA been pure virus nucleic acid, one could not postulate a transfer of genes from the mouse cell. The preparation, however, also contained cellular DNA from the mouse embryo cultures.

Transplanted hamster polyoma virus-induced tumors appear to be free of the virus. It could not be recovered by culturing the tumor tissue in mouse embryo cells, nor could the virus be released by induction methods used for releasing prophage (40, 41). From these observations and because of failure to find evidence of necrotizing virus in hamster embryo cultures and in primary hamster tumors (from a study of histological sections of the tumors),

it appears that the virulent virus capable of causing cell lysis has become converted to a temperate virus in which one of the following may have occurred: (*a*) The virus may be present as a closely integrated provirus that is not released by the usual induction procedures. (*b*) It may be present as an incomplete or non-infectious virus so that it is not possible to demonstrate it by tissue culture methods. (*c*) It may have taken part in the initial cell transformation as a chemical carcinogen, and not be passed to the daughter cells.

In the mouse cells, transformation to the neoplastic state by the virus does not require the transfer of genes or of proteins to establish tolerance. Here it can be explained as a process of conversion similar to the change produced by the Rous virus on chick cells. The age of the infected cells appear to be of particular importance, as transformation rarely results if the cells are not more than 24 hr. old.

Neoplastic Transformations of Cells in Animals With Human Tumor Materials

Recent work with purified nucleic acids, crude tissue extracts, etc., derived from human neoplasms have lead to results that merit further investigation. Schwartz *et al.* (42) reported a decrease in the latent period for the development of leukemia in strain AKR mice when they were injected with filtrates prepared from human leukemic brains obtained at autopsy. Twenty-two per cent of 326 adult mice, inoculated intracerebrally or intraperitoneally with the brain filtrate, developed leukemia before they reached the age of 22 weeks instead of the usual 10 to 12 months. In many, the latent period after injection was only two to four weeks. Bergoltz (43) has described a 34 per cent incidence of leukemia in mice inoculated with saline extracts prepared from human leukemic tissues and an even higher incidence with extracts from human sarcomas. He also has described the cultivation on chick chorioallantoic membrane of a "leukemia factor" obtained from the blood of patients with acute lymphatic leukemia and from the brains of patients with myelogenous leukemia who had died. Chorioallantoic fluids inoculated into mice caused a leukemia incidence of 17.7 per cent as opposed to 2.7 per cent in mice injected with chorioallantoic fluids from uninoculated eggs. Two of the mice receiving material from human leukemic tissues developed tumors of the parotid glands.

Burton *et al.* (44) have reported the induction of parotid gland tumors and other neoplasms in strain C_3H mice that were inoculated with a "tumor factor" recovered from purified concentrates prepared from the serum and red blood cells of patients with Hodgkin's disease. The "tumor factor" also produced a tumor in the fruit fly, *Drosophila melanogaster*. Serum from non-neoplastic donors did not produce tumors in either mice or flies.

In studying the oncogenic properties of cell-free filtrates of human tumors, Grace *et al.* (45) found that filtrates from a variety of human tumors, when inoculated into newborn Swiss mice of their colony, produced mammary adenocarcinomas in from 0 to 20 per cent of the pregnant females receiving

the different preparations. The same tumor was also seen in 3 of the inoculated male mice and in 10 non-pregnant females. This tumor was not observed in their control mice.

Activation of latent oncogenic viruses in the host.—In the Schwartz and Burton experiments the strains of mice used are known to carry tumor-inducing viruses. The strain of AKR mice used by Schwartz *et al.* has been found to carry a leukemia virus (46), and the strain of C_3H_f mice used by Burton *et al.* is the Gross subline which has been reported to have a high incidence of latent infection with polyoma virus (12). Schwartz and colleagues interpret their results as resulting from a leukemogenic substance, probably a virus, in the brain filtrates that activates a process which would have occurred naturally at a later date. In the experiments of Burton *et al.* the control mice did not develop tumors. The tumors observed in the mice that received the "tumor factor" were of the polyoma virus spectrum. They interpret their results not as an activation of tumor viruses in the mice, but as an induction with the injected "tumor factor." Experiments of Stewart *et al.* (47) have shown that maternal antibodies protect the offspring of polyoma virus-infected mothers, preventing them from developing tumors though exposed to the virus. This would account for the absence of tumors in the mouse colony that carried a latent polyoma virus infection.

Bergoltz used mice with a very low incidence of spontaneous leukemias for his experiments and therefore felt justified in his conclusions that the leukemias which occurred had been induced with the inoculum. Lieberman & Kaplan (5) have reported the recovery of a leukemia virus from radiation-induced leukemias in strain C57 black mice that have a very low incidence of spontaneous leukemia but are very susceptible to leukemogenesis by radiation. They concluded that in this low leukemia strain radiation activates a latent leukemia virus that would remain inactive under natural conditions. The presence of a latent leukemia virus in Bergoltz's mice was not ruled out; since two of his mice developed parotid gland tumors after inoculation of the human material, the possibility should be considered that his mouse colony also harbored a latent polyoma virus infection.

The four different experiments cited wherein tumor induction was reported as a result of inoculating extracts or filtrates from human neoplastic material could have resulted from a specific factor, possibly a virus itself not oncogenic, which was capable of activating latent tumor viruses in the mice. This was considered as a possibility by Grace *et al.* in their experiments.

Host specificity of tumor viruses.—Although some of the tumor viruses can be made to produce neoplasms in foreign hosts by a process of adaptation, they are usually specific. The Bittner milk agent which produces mammary adenocarcinomas in mice is considered to be highly specific, producing tumors in only certain genetic strains (48). Polyoma virus has been shown to have a greater variability in producing tumors in various species, but these have been limited to rodents. Newborn monkeys inoculated with the virus have remained free of neoplasms. These have now been under observation two and

four years (49). Svet-Moldavsky (50) studied the pathogenicity of Rous sarcoma virus for mammals and reported that rats inoculated during embryonic life or shortly after birth developed cysts and hemorrhagic lesions in the organs 6 to 45 days after inoculation.

McClaren and his co-workers have demonstrated the non-specificity of purified nucleic acids of polio virus (51). The nucleic acid could infect a cell previously resistant to the whole virus, and could replicate in the cell producing complete viruses which, like the original virus, were non-infectious for the resistant cells. Lacour *et al.* (52) reported that a purified nucleic acid derived from a human lymphoma produced tumors in two mice that were inoculated with it intraperitoneally. They were unable to repeat their results. It is conceivable that such a loss of specificity as they describe could occur if a purified nucleic acid were derived from a human oncogenic virus rather than from the tumor tissue. A given human tumor at a certain stage in its development may have present, as shown with polyoma virus-induced tumors, a large amount of the inciting virus. At this stage the viral nucleic acid from the tumor may be capable of producing tumors in a foreign host, whereas the whole virus would not because of its high specificity.

Potentialities of a Specific Tissue Culture System

Primary tissue cultures of human embryonic cells grown in the absence of antibodies, such as may be encountered in human serum frequently used for growing out cells, may provide the best medium for detecting oncogenic viruses or other substances capable of inducing neoplastic transformations from human material. The transformation of human embryonic cells with a substance from human neoplasms was recently reported by Stewart & Irwin (53). It appears that such a tissue culture system may have great potentialities for studies on the mechanisms of oncogenesis of human tissues.

HEMATOLOGY: IRON METABOLISM[1]

By Ernest Beutler, M.D.[2]

Departments of Medicine, City of Hope Medical Center, Duarte, California, and University of Southern California School of Medicine, Los Angeles, California

Today, the importance of iron in human nutrition is widely appreciated. difficult to realize that only 30 years ago very little was known about metabolism, and iron deficiency was not recognized as a clinical entity. ng the first three decades of this century, chlorosis was commonly re-:d as a form of neurosis, attributable to tight corsets, endocrine disturb-, toxemia, or a variety of other causes (4, 7, 75, 105, 115). Those prin-of iron metabolism that we now regard as basic were totally unknown. etween 1930 and 1950, tremendous strides were made in the under-'ing of iron metabolism. However, re-evaluation of some of the con-ns which have been reached as a result of the work of pioneers in this n the 1930's and 1940's, has been necessary in the past decade. Much of rly work has stood the test of time, but reappraisal of some of the con-that had been developed and widely accepted has shown that, at times, speculations were accepted as facts, important basic principles were d on uncontrolled studies carried out on a few experimental animals, iagrams of hypotheses were sometimes accepted as basic biologic

41. Stewart, S. E. (Unpublished data)
42. Schwartz, S. O., Schoolman, H. M., Szanto, P. B., and Spurrier, W., *Cancer Research*, 17, 218–22 (1957)
43. Bergoltz, V. M., *Neoplasma*, 5, 337–47 (1958)
44. Burton, L., Friedman, F., Kassel, R., Kaplan, M. L., and Rottino, A., *Trans. N. Y. Acad. Sci.*, 21, 700–7 (1959)
45. Grace, J. T., Mirand, E. A., and Mount, D. T., *Arch. Internal Med.*, 105, 482–91 (1960)
46. Gross, L., *Proc. Soc. Exptl. Biol. Med.*, 76, 27–32 (1951)
47. Stewart, S. E., Eddy, B. E., Irwin, M., and Lee, S., *Nature*, 186, 615–17 (1960)
48. Andervont, H. B., and Dunn, T. B., *J. Natl. Cancer Inst.*, 14, 317 (1953)
49. Stewart, S. E., and Eddy, B. E. (Unpublished data)
50. Svet-Moldavsky G. J., *Acta Virol.* 2, 1–6 (1958)
51. McClaren, L. C., Holland, J. J., and Syverton, J. T., *J. Exptl. Med.*, 109, 475–86 (1959)
52. Lacour, F., Lacour, J., Harel, J., and Huppert, J., *J. Natl. Cancer Inst.*, 24, 301–28 (1960)
53. Stewart, S. E., and Irwin, M., *Cancer Research*, 20, 766–67 (1960)

HEMATOLOGY: IRON METABOLISM[1]

By Ernest Beutler, M.D.[2]

Departments of Medicine, City of Hope Medical Center, Duarte, California, and University of Southern California School of Medicine, Los Angeles, California

Today, the importance of iron in human nutrition is widely appreciated. It is difficult to realize that only 30 years ago very little was known about iron metabolism, and iron deficiency was not recognized as a clinical entity. During the first three decades of this century, chlorosis was commonly regarded as a form of neurosis, attributable to tight corsets, endocrine disturbances, toxemia, or a variety of other causes (4, 7, 75, 105, 115). Those principles of iron metabolism that we now regard as basic were totally unknown.

Between 1930 and 1950, tremendous strides were made in the understanding of iron metabolism. However, re-evaluation of some of the conclusions which have been reached as a result of the work of pioneers in this field in the 1930's and 1940's, has been necessary in the past decade. Much of the early work has stood the test of time, but reappraisal of some of the concepts that had been developed and widely accepted has shown that, at times, mere speculations were accepted as facts, important basic principles were founded on uncontrolled studies carried out on a few experimental animals, and diagrams of hypotheses were sometimes accepted as basic biologic truths.

It is the purpose of the present review to re-evaluate our state of knowledge in several areas in the field of iron metabolism. Because of the limitations of space, only certain aspects of iron metabolism can be discussed. We shall consider how iron traverses the intestinal mucosa to enter the blood stream and what regulates the amount absorbed. Special attention will be paid to increases in our understanding of the most common disorder of iron metabolism-iron deficiency. The relative merits of various tests which may be used to establish this diagnosis will be discussed and the evidence for tissue involvement in iron deficiency will be presented. Several reviews covering various aspects of iron metabolism have appeared in recent years, including some topics not covered in the present review (8, 15, 30, 44, 52, 68, 87, 123).

The Mechanism and Regulation of Iron Absorption

In 1936, McCance & Widdowson (101) demonstrated that iron is not excreted by the body. These studies have been amply confirmed by more recent evaluation of iron balance using Fe^{59} or Fe^{55}, which have demonstrated that losses of iron from the body are, indeed, minute (29, 50, 54). Thus, regulation of absorption must serve as the main means of maintaining

[1] The survey of the literature pertaining to this review was concluded in July, 1960.

[2] This study was supported in part by Public Health Service Grant No. H 5618.

a normal quantity of iron in the body. Until very recently, it was widely accepted that the amount of iron in the body was regulated by the "mucosal block mechanism." This mechanism was believed to operate by virtue of the fact that mucosal cells became saturated with ferritin when iron was fed. Thus, it was thought that for a period of time after iron had been fed, no further iron could be absorbed. This concept was based on studies published by Hahn *et al.* (72) in 1943. These authors carried out essentially uncontrolled experiments in three dogs. They observed that when a large dose of iron was followed within 1.3 to 6 hr. by a dose of radioiron, less radioactivity appeared in the erythrocytes than might have been "expected" in four of five cases. They concluded that the first dose of iron had "blocked" the second dose of iron. Inconsistencies were explained on the basis of uneven contact of mucosa with the iron solution. These authors failed to take into account, however, that for radioactivity to appear in the red cell it was not only necessary for iron to be absorbed from the gastrointestinal tract, but also for it to be utilized for erythrocyte formation. Since not only the bowel mucosa but also the marrow had been flooded with large amounts of iron, the "blocking" of radioiron may have occurred not at the bowel mucosa, but actually at the bone marrow level. In point of fact, more recent studies, using more acceptable techniques, have invariably demonstrated that although some decrease in iron absorption may occur when iron is given, no absolute block of mucosal absorption is produced by the administration of iron (33, 40, 119, 138). However, the experiments of Hahn *et al.* have formed the basis of a great deal of further experimentation and speculation on the mechanism of iron absorption.

Granick studied guinea pigs which had been fed relatively huge quantities of iron. Treatment of mucosal scrapings from such animals with cadmium sulphate resulted in the the formation of crystals having an appearance characteristic of ferritin (64). Accepting the concept of a "mucosal block," Granick postulated that the amount of ferrous iron moving into a mucosal cell might be regulated by a mucosal-blocking mechanism related directly to the level of ferrous iron in the cell and indirectly to the ferritin concentration (63, 65). He suggested that, in the mucosal cell, ferrous iron combined with apoferritin to form ferritin and that the amount of ferrous iron which left the mucosal cell and entered the blood stream depended on the relative redox level of the cell. This, in turn, was believed to be influenced by the decrease of oxygen tension of the blood stream in anemia. No evidence was presented that in anemia a decrease in the oxygen tension of the bowel mucosa actually occurs. In considering the possible validity of this postulated mode of absorption, it is disturbing to note that Granick's own data show that the increase in ferritin concentration of the bowel does not begin for 4 to 5 hr. (64) or 3 or 4 hr. (63) after feeding, and that the maximum level is reached after 7 hr. It is well known that, at least in human subjects (42, 61, 112) and in dogs (72), the peak of plasma iron levels is achieved between 1 and 3 hr. after administration of iron. It is reasonable to suppose

that in small experimental animals the peak is reached even sooner. If, however, ferritin bears a precursor relationship to plasma iron, the peak in plasma iron levels would be expected to follow and not to precede the peak of mucosal ferritin concentration. The finding that ferritin is formed in mucosal cells after the administration of iron to experimental animals has been confirmed (56, 64, 138) but it must at present be considered uncertain whether mucosal ferritin is a true intermediate in the transport of iron from the lumen of the bowel to the blood stream or whether it has merely a storage function (30). It has been suggested that iron chelates present in the lumen of the bowel pass through the mucosa without alteration (113). There is no convincing evidence supporting this point of view.

Attempts have been made to devise methods for the study *in vitro* of the passage of iron through the intestinal mucosa. Brown & Justus (34) bathed everted pouches of rat intestines in a buffer system containing either ferric citrate or ferrous ascorbate gelatine labeled with Fe^{59}. Their results suggest that the uptake of iron by such pouches was a passive process, not appreciably affected by changes in pH, metabolic inhibitors, or added substrate. There was little transfer of iron into the pouch fluid. In a more recent study, Dowdle *et al.* (48) claim to have demonstrated active transport of iron across the bowel, using the everted pouch technique. Inhibition was achieved by the use of azide, fluoride, malonate, cyanide, 2,4 dinitrophenol, and by anoxia. It is suggested by these workers that the failure of Brown & Justus to demonstrate active iron transport might be attributed to the fact that these authors used the middle and lower portion of the bowel in most of their studies, while in Dowdle's investigations, the upper portion of the bowel was employed. However, upper portions of the bowel were used in at least some of the studies reported by Brown & Justus (34). Unfortunately, comparison of the data of these two groups is not possible because of the differences in units employed. If confirmed, the results of Dowdle and his colleagues could be of value in the study of the mechanism of iron transport. However, it is uncertain whether the transport they have observed is related to the physiological mechanism of iron absorption. It is of interest in this respect that bowel anoxia, believed by some to represent a direct stimulus to absorption (65, 99), inhibits transport of iron in their system.

While there is little direct experimental evidence to aid us in our understanding of how iron traverses the mucosa of the bowel, certain investigators have given their attention to the mode of entry of iron into other cells, particularly liver cells. Such data are worthy of serious consideration in relation to the problem of iron absorption, since iron uptake and release from storage sites may be controlled by mechanisms similar or identical to those regulating iron absorption (40). Saltman and others have investigated the entry of iron from ferric ammonium citrate into rat liver cell suspensions and liver slices. They found that rat liver slices had the capacity to accumulate exogenous iron against a concentration gradient (116). They suggested that iron passes through the cell membrane in both directions passively. Once inside the cell,

the iron was bound in some manner to an iron-binding entity. It was suggested that this entity could be ferritin. Studies with metabolic inhibitors indicated that the process was independent of the cell's respiratory energy (1, 117). Initial iron accumulation was found to involve the nucleus (2). The synthesis of the aminn acid chains of ferritin within the liver cell has been studied by Loftfield & Eigner (98), and appears to be an active metabolic process.

In Granick's early studies of apoferritin (66), he found that ferritin could be regenerated from apoferritin by mixing it with an irnn-containing brown mother-liquor remaining after crystallization of ferritin. Other iron compounds studied were not incorporated into apoferritin to form ferritin. However, Bielig & Bayer (26) were able to form ferritin by passing air through a solution containing apoferritin, ferrous ammnnium sulfate, and bicarbonate. Loewus & Fineberg (97) were also able to achieve synthesis of ferritin from ferric ammonium citrate and apoferritin in the presence of a boiled liver extract. Ascorbic acid could substitute for this extract in bringing about the incorporation of ferric iron into rat apoferritin but not into horse apoferritin. The incorporation of plasma-bound iron into hepatic ferritin has also been investigated by Mazur *et al.* (99). When liver slices were used, substrates for oxidative enzymes such as citrate, oxalacetate, malate, and fumarate were found to increase the incorporation of iron into ferritin. Inhibitors such as iodoacetamide, cyanide and arsenite, and anoxia were found to decrease incorporation. Differences between these findings and those of Saltman *et al.* (1, 117) may be based on differences of iron transport between ionic and plasma-bound iron. Such differences have already been demonstrated by Jandl *et al.* (82) in the case of iron transfer to reticulocytes. To reconcile tbe differences between their findings and those of Saltman *et al.* (1, 117), Mazur *et al.* (99) studied the incorporation of ionic and plasma-bound iron into liver slices, both as ferritin and as total liver uptake. Inhibition by anoxia and metabolic inbibitors was nbserved only when iron incorporation into ferritin was considered and was best demnnstrated when the iron was plasma-bound. The incorporatinn nf plasma-bound radioiron into recrystallized ferritin in tbe presence nf rat liver hnmogenates was also investigated. It is perbaps unfortunate that apnferritin, the natural substrate for iron incorporation, was not used. These studies led tn the conclusion that both ascorbic acid and ATP were required fnr the transfer nf plasma-bound iron to ferritin. ATP and ascorbic acid appeared to be able to do this even in the absence of any substance from liver. It was believed that the incorporation of iron into ferritin might be dependent upon energy because of the requirement for ATP. The quantity nf irnn incorporated into ferritin in Mazur's studies was quite small, and it is nnt entirely clear whether the incorporation of iron into ferritin observed in this system represents the physiological means by which iron is bound tn apoferritin. Similarly, the physiological significance of the observatinns nf Loewus & Fineberg (97) and nf Bielig & Bayer (26) is somewhat uncertain at present. It has generally been assumed

that iron enters the cell either in ionized form or perhaps bound to a biological chelating agent of small molecular size. However, in studies of the uptake of iron by erythroblasts, using electron microscopic techniques, Bessis & Breton-Gorius (6) have concluded that iron may enter the developing erythroblasts in the form of ferritin itself through the process of pinocytosis. It is conceivable that other body cells may participate in iron transport by means of a similar mechanism.

Bielig & Bayer (25) have shown that iron may be removed from ferritin by sodium dithionite, ascorbic acid, glutathione, and DPNH, in that order of efficiency. The release of iron from ferritin has also been investigated by Green & Mazur (67). The operation of the xanthine oxidase system was found to result in release of iron from purified horse spleen ferritio, as had been suggested by the studies of Tanaka (127). It should be pointed out, however, that only minute amounts of iron, amounting to 0.4 per cent of the total amount present, were mobilized in the studies they have reported. In their earlier studies, Green & Mazur (67) reported that the mobilization of iron from ferritin by the xanthine oxidase system was enhanced by oxygen. If iron absorption is controlled by bowel anoxia, this result would be quite unexpected. In more recent studies, however, (100) the authors have concluded that the earlier findings arose from a technical error, namely, that an aged xanthine oxidase preparation had been used. Greater release of iron from ferritin was obtained under N_2 than under O_2 wheo fresh enzyme was employed. Mazur *et al.* (100) have also investigated the problem of whether the xanthine oxidase system is physiologically active in release of iron from the stores. They had found that mobilization of iron stores by inducing hemorrhage shock in dogs was accompanied by a rise in blood levels of uric acid, the product of xanthine oxidation. They also demonstrated that the infusion of hypoxanthine, xanthine, adenylic acid, and inosine resulted in a rise in plasma iron and uric acid levels. Uric acid, glycine, butyric acid, and adenosine failed to cause a rise in the plasma and iron level. Cheney & Finch (40) have observed slightly increased iron absorption in experimental animals given inosine parenterally, but not orally. They pointed out that the magnitude of increased iron absorption noted in iron deficiency and hemochromatosis is so much greater as to suggest that a different mechanism may be operative. The data relating iron release and xanthine oxidation are interesting and should stimulate further study of the possible role of this system in the release of iron from ferritin. However, it cannot be regarded as demonstrating conclusively that xanthine oxidation plays an important physiological role in the release of iron from ferritin or in the absorption of iron. Other substances, such as cysteine, appear to be capable of inducing loss of iron from ferritin *in vivo* (138) as well as *in vitro*.

In the past few years a number of investigators have undertaken to reinvestigate the entire problem of the regulation of iron absorption. The results of earlier isotope studies (72), in which it was assumed that all iron absorbed was incorporated into hemoglobin, are suspect because it has been

demonstrated that this assumption is incorrect (49). Three isotope techniques are available for the accurate determination of the amount of iron absorbed. The first of these is the measurement of the radioactivity of the stools for a period of days following the administration of radioiron orally (31, 49, 119). This is a sound method for the measurement of iron absorption, but has certain serious inherent limitations. Because the greatest part of the iron ingested is in the excreted portion, relatively small errors in the stool collection are greatly magnified in the calculation of the percentage of iron absorbed. For example, if an experimental subject excreted 90 per cent of the ingested dose, a one per cent error in the counting or collection of the stool sample would result in a 10 per cent error in the estimated quantity of iron absorbed. This problem may be overcome, in part, by feeding such small doses of iron that a relatively large proportion is absorbed (28). However, problems of obtaining accurate stool collections are well known. A second method of measuring iron absorption is to use two different isotopes of iron, Fe^{55} and Fe^{59}, giving one isotope by the oral route, the other by the intravenous route (31, 118). The percentage of the oral dose which is incorporated into the circulating red cell mass is determined. It is then possible to estimate accurately the proportion of the oral dose that has been absorbed by taking into account the proportion of the intravenously administered isotope that appears in the red cells. A basic assumption in these calculations is that the distribution of iron entering the body through the gastrointestinal tract is identical to that which is injected directly into the peripheral venous circulation. Under most circumstances, this would seem to be a valid assumption. Indeed, studies of the iron absorbed, by means of this method, correlate well with that determined by other acceptable techniques. The counting of Fe^{55} presents certain technical problems. These have been decreased to some extent by the development of a method of counting both isotopes in a scintillating phosphor developed by Dern & Hart (45).

The simplest method of measuring iron absorption is by total body counting. While ashing of the entire animal and counting of an aliquot may be carried out, improved instrumentation makes simpler approach possible. Radioiron is given to the experimental subject by the oral route and a total body count is carried out against a standard. A second total body count is carried out at some later date at which time all of the iron has been cleared from the gastrointestinal tract and is compared with the same standard. The fraction of radioactivity left in the body will represent the fraction of the dose of iron absorbed. In using this method, it is necessary to demonstrate that with the detection equipment employed, the same number of counts are obtained with a given quantity of iron deposited in the stomach and upper gastrointestinal tract as when it is distributed over the entire body. This has been demonstrated to be the case with mice (91) and rats (19, 53) with available counting equipment. If this condition is not met, an

alternate method is also available. One may administer a dose of n microcuries of Fe^{59} via the oral route and count the subject after several days. Immediately following, n microcuries are given intravenously and the subject is counted again. The difference between the last two counts represents the number of counts detected from n microcuries of Fe^{59} distributed throughout the blood stream and reticuloendothelial system. The percentage absorbed can then be readily estimated from the first count taken.

Using improved techniques for the measurement of iron absorption, certain basic facts have become well established. These are summarized in Table I. Anemia, produced by bleeding or by hemolytic agents, greatly

TABLE I

A. Factors which augment iron absorption
- I. Anemia
  - a. Induced by bleeding (31, 72, 91)
  - b. Induced by hemolysis (31, 91, 125)
- II. Anoxia (91)
- III. Cobalt administration (91)
- IV. Iron deficiency (19, 53, 108)

B. Factors which diminish iron absorption
- I. Transfusion polycythemia (31, 91)
- II. Iron overload (31, 91, 108)
- III. Bone marrow aplasia (31, 91, 107)

augments the absorption of iron (31, 72, 125). The administration of cobalt (91), hypoxia (91), and iron depletion without anemia (108) similarly augment the absorption of standard doses of iron. Bleeding causes increased absorption of iron even if the iron is quantitatively replaced by the parenteral route (13). On the other hand, transfusion polycythemia depresses the absorption of iron (31, 91), as does loading with parenteral iron (31, 91, 108). In human patients with aplastic anemia, decreased iron absorption has been described (107), but what probably represents augmented absorption has also been measured (96). It is difficult to evaluate clinical data because of the retarding effect of increased iron stores on iron absorption. Serial studies of absorption in one patient with aplastic anemia showed a decrease in iron absorption after transfusion (96). Available evidence suggests that breathing an atmosphere of increased oxygen content does not retard the absorption of iron (102). Iron transfer across the bowel frequently is against a gradient (31). It is not enhanced by the administration of plasma containing large amounts of iron-binding protein (19). It is not a process of simple diffusion, but rather has the characteristics of an active, finely regulated process.

It seems clear, however, that the regulation of iron absorption cannot be

explained entirely on the basis of anemia-induced bowel anoxia. If the state of oxygenation of the bowel depended on the hemoglobin level, and if it were the sole determinant of the rate of iron absorption, anemic subjects should absorb increased amounts of iron, regardless of their state of marrow activity. Further, breathing an atmosphere of pure oxygen should diminish, at least to some extent, the absorption of iron. If anemia-induced bowel anoxia were the sole regulator of iron absorption, then iron-deficient subjects without anemia should have normal, instead of increased iron absorption, and loading with iron should not diminish iron absorption. Another theory which does not depend on the effects of bowel anoxia has recently been proposed. It is based on the observation that there is excellent correlation between the degree of marrow activity and the rate of iron absorption (32, 103). This attractive theory suggests that either the marrow is under the same humoral control as iron absorption, or that a humoral substance is produced in the process of marrow erythropoiesis that stimulates the absorption of iron by the bowel. Injection of experimental animals with erythropoietin has failed to result in increased iron absorption (91). Further plasma from iron-deficient animals does not stimulate iron absorption in normal animals (19). Finally, this theory alone fails to explain the increased iron absorption in non-anemic subjects with depleted iron stores (108), subjects in whom marrow activity is normal, nor the decreased iron absorption which occurs with iron loading.

It has also been suggested that perhaps a deficiency of iron enzymes in mucosal cells may cause enhanced iron absorption (58). It is of interest, in this respect, that xanthine oxidase, believed by Mazur *et al.* (100) to play an important role in iron transport, contains iron (55). While a deficiency of iron enzymes could explain the increased iron absorption occurring in iron deficiency, it would fail to explain satisfactorily the increased iron absorption occurring after another stimuli, particularly after the administration of phenylhydrazine. In addition, we have found that if iron is quantitatively returned parenterally to rats that have been acutely bled or is given to them just prior to bleeding, the increase in iron absorption is just as great as in those in which no replacement of iron is attempted (13).

To our knowledge, no single theory has been proposed which satisfactorily explains all the phenomena listed. It seems likely that iron absorption is under dual control. The size of iron stores would seem to be one parameter which influences iron absorption. Either bone marrow activity or anemia could be additional parameters. By what pathways iron absorption is stimulated remains unknown. Investigations of the secretions of the gastrointestinal tract have failed to reveal a substance which, secreting into the lumen, enhances the absorption of iron (13). Greater understanding of just how iron traverses the intestinal mucosa to enter the blood stream might provide greater insight into the mechanism of control of iron absorption.

THE DIAGNOSIS OF IRON DEFICIENCY

Severe iron deficiency anemia.—The diagnosis of severe iron deficiency anemia does not ordinarily present a difficult problem. In well-developed iron deficiency, when the hemoglobin level of the blood is below 8 or 9 gm. per cent, the corpuscular constants are low, the red cells are hypochromic and microcytic, and aniso- and poikilocytosis are noted on the smear. In the vast majority of cases, the diagnosis of severe iron deficiency anemia can be made on the basis of these findings and on the basis of the clinical history of blood loss. The rapid response to the administration of iron gives final confirmation of the correctness of the diagnosis. Occasionally, differentiation from other hypochromic microcytic anemias becomes necessary, particularly the thalassemia syndromes, the occasional cases of anemia caused by chronic infection or malignancy, the rare cases of pyridoxine-responsive anemia, or sidero-achrestic anemia. In these instances, the determination of the plasma iron and the iron-binding capacity may be very helpful. The plasma iron is depressed, not only in iron deficiency, but also in patients with the anemia of chronic infection or malignancy (15, 24, 38, 39, 76, 111, 131), and may be somewhat low in thalassemia minor (24, 120). However, the unsaturated iron-binding capacity is almost invariably greatly increased in severely iron-deficient patients (24, 111, 131); such increases are not observed in the other conditions from which iron deficiency must be differentiated. Flat iron-tolerance curves have been described and have been attributed to "decompensation" of the mucosal mechanism for iron absorption in severe anemia (129). It seems equally likely, however, that the very rapid clearance of iron from the plasma, which occurs in severe iron deficiency, may account for the failure of the plasma iron to rise to higher levels upon the administration of oral iron to severely iron-deficient patients.

Unlike the bone marrow of patients with anemias from other causes, the bone marrow of iron-deficient patients is usually devoid of stainable iron, unless they have received iron by the parenteral route or have recently received blood transfusions (15, 21, 24, 43, 74, 80, 109, 110, 124, 132, 134, 135, 136, 139). Similarly, normoblasts containing iron granules (sideroblasts) are absent from marrow smears of iron-deficient patients (15, 24, 47, 74). While stainable marrow iron represents available storage iron, it has recently been suggested that the presence of sideroblasts more accurately reflects the immediate availability of iron for hemoglobin synthesis (74). Thus, patients who have become anemic because of acute blood loss may have relatively intact marrow iron stores but sideroblasts may have disappeared from the marrow.

Mild iron-deficiency.—The diagnosis of mild iron deficiency may present more of a clinical problem than does a diagnosis of severe iron deficiency. This disorder must be differentiated not only from the thalassemia syndromes, from the anemia of chronic infection or malignancy, but also from physiological variants in which the hemoglobin level happens to fall in the

lower portion of the normal distribution curve, i.e., in subjects who are not truly anemic.

In mild iron deficiency, mild hypochromia and microcytosis may be present, but it is not unusual for the red cell indices to be normal (16, 22, 78, 123). Even in the presence of normal indices, however, careful examination of the stained smear of blood may demonstrate the presence of a few hypochromic microcytes or poikolocytes (16). The plasma iron and iron-binding capacity are probably of somewhat less value in mild iron deficiency than in severe. The reports of some authors seem to show a consistent decrease in plasma iron and increase in unsaturated iron capacity, even in the mildly anemic or non-anemic iron-deficient patients (131). However, we have observed normal plasma iron and iron-binding capacity values in some patients with mild iron deficiency (22, 24), and it has recently been reported that in subjects in whom the iron stores were deliberately depleted by phlebotomy, plasma iron levels were, if anything, increased (108).

The iron-tolerance curve has had considerable popularity, especially in certain European centers, in the diagnosis of mild iron deficiency (42, 59, 60, 77, 83 to 86; 112, 128, 131). The theoretical objections to the use of this test for the diagnosis of iron deficiency have been discussed (15, 57, 59). The shape of the iron-tolerance curve obviously depends on a combination of factors, including not only the quantity of iron absorbed but also the rate of iron absorption and the rate of iron clearance from the plasma to both marrow and storage sites. Clearly, patients with iron deficiency arising from malabsorption would have flat iron-tolerance curves in spite of being iron-deficient (42). Standards for the interpretation of iron-tolerance curves have been poorly defined. However, a recent study of iron tolerance curves suggests that, at least in many mildly iron-deficient patients, high tolerance curves are achieved (131). It is of interest to note that all of these patients also had high plasma unsaturated iron-binding capacities present. It was therefore concluded that the iron-tolerance curve presents no advantage over the determination of the plasma iron and iron-binding capacity, a technically simpler procedure (131). Recently, the use of radioiron curves has been proposed as a means for evaluating the iron stores (94). This claim has not been documented by any data and, in point of fact, study of the potential of this method for diagnosing iron deficiency has yielded disappointing results (14, 24). The use of an iron chelate has also been found to be useful in assessing the size of the iron stores (89, 90). Further work will be required to establish the practical value of this method. The free erythrocyte protoporphyrin probably does not rise sufficiently in mild iron deficiency to be of diagnostic value (38, 78, 121).

The examination of the bone marrow for stainable iron has been the most reliable means of diagnosing mild iron deficiency anemia (24). This method has several drawbacks, however, First of all, it is necessary to perform a bone marrow puncture to obtain material for examination. Secondly, meaningful observations can be made only by well-trained observers, since

artifacts commonly occur in preparations and must be differentiated from true storage iron. Third, it is likely that this method is somewhat overly sensitive, since very small amounts of storage iron may readily be missed in the small fragments of marrow available for examination. Furthermore, it is entirely possible that iron stores may be completely depleted without affecting hemoglobin mass or any other type of functionally active iron. It is hoped that eventually one of the simpler means of evaluating the size of the iron storage pool may be proven sufficiently reliable to be of greater value than bone marrow iron estimation.

The Tissue Changes in Iron Deficiency

Until recent years, full attention in iron deficiency was focused upon the changes in the blood. Aside from excellent clinical descriptions of tissue changes occurring in iron deficiency, the changes in tongue, esophagus, and fingernails, scant attention was paid to any other alterations of metabolism which iron deficiency might cause. Thus, the symptoms of iron deficiency have commonly been ascribed to the anemia (122); other iron compounds of the body have been ignored probably because of their minute quantity and because they were difficult to measure. However, even clinical studies of many years ago suggest that the symptoms of iron deficiency might occur in the absence of anemia (3, 34, 92, 104, 105, 115). In iron-deficient patients, the response of symptoms to iron therapy preceded any significant rise in the hemoglobin level (34, 62, 76). Further, it is difficult to explain the tissue changes which occur in iron deficiency on the basis of the anemia alone. The esophageal changes, which may occur in iron deficiency even in the absence of anemia, are reversed by the administration of iron (114, 133). Similarly, in young men with iron-deficiency anemia, gastric achlorhydria will respond to iron therapy (37, 51, 95), although in some groups of older persons such a response does not appear to be seen (51, 93).

In ignoring the possible role of tissue enzymes in the clinical course of iron deficiency, an early observation of Cohen & Elvehjem (41) suggesting that there might be a decrease in the cytochrome-*c* content of tissues from iron-deficient rats, had been forgotten. On the other hand, the statement made by Hahn & Whipple (70, 71, 73) that tissue iron enzymes were "inviolate" even when the body's need for iron was great, has been cited frequently, although it lacks experimental support. Vannotti (130) has mentioned that the cytochrome-*c* level of iron-deficient experimental animals was low, but the first quantitative measurements in iron-deficient experimental animals were reported by Gubler and associates in three iron-deficient piglets (69). These authors found the levels of cytochrome-*c* and myoglobin to be greatly reduced in the tissues of these animals. Recently, we have undertaken a systematic evaluation of iron enzymes in larger numbers of iron-deficient rats. It has been found that cytochrome-*c* was readily depleted from liver and kidneys of iron-deficient rats (10, 18), while the catalase content of iron-deficient rat livers, rat red cells, and human red

cells remained remarkably unaffected by iron deprivation (17, 18). Intermediate degrees of depletion were found to occur in the iron enzymes, cytochrome oxidase (9), and succinic dehydrogenase (20). Iron deficiency was found to exert an interesting effect on the activity of aconitase, an enzyme that appears to require iron as a cofactor (46). Aconitase activity of iron-deficient rat kidney was found to decline markedly, even with relatively mild degrees of iron depletion (11). Treatment of iron-deficient animals with iron restored kidney aconitase to normal. It was not possible, however, to reactivate the enzyme *in vitro* by the addition of iron. Apparently the apoenzyme itself was not synthesized by the organism when its cofactor was not available. This situation is perhaps analogous to the postulated dependence of apoferritin synthesis on the presence of iron (63, 64), and on the apparent dependence of the synthesis of the globin portion of hemoglobin on the presence of heme (12).

Additional evidence has accumulated that iron compounds other than hemoglobin and storage iron are affected by iron deficiency. Kampschmidt *et al.* (88) have confirmed that iron-deficient rats have decreased tissue cytochrome-*c* levels. The examination of thermally induced sweat showed a marked decrease in the iron content, both of the cell-rich and cell-free portion (79). The study of oral mucosal cells of iron-deficient subjects has shown morphological abnormalities which are independent of the degree of anemia (27, 81). How enzymatic changes occurring in iron deficiency affect the organism remains obscure.

Preliminary studies in our laboratory of the oxygen consumption of slices of iron-deficient kidneys with marked cytochrome-*c* depletion showed no decrease in oxygen consumption. Similarly, the oxygen consumption of exercising iron-deficient human subjects was not influenced appreciably by iron therapy (23). Thus, it may well be that the oxidative iron enzymes studied do not, even at their decreased levels in iron-deficient subjects, represent the limiting step in the involved metabolic pathway. Iron participates in the economy of the body in a variety of ways. Aside from its role as a part of certain enzymes having to do with tissue oxidations, iron may be an integral part of other enzymes and the ferrous ion may participate as a cofactor in many enzymatic reactions (5, 36, 55, 106, 126). It is entirely possible that it is interference with one of these functions that may be critical in the physiological effect of iron deficiency. The growth changes that occur in iron-deficient subjects suggest that the lack of iron may interfere with certain synthetic processes. In *Mycobacterium smegmatis* it has been suggested that decreased growth in iron-deficient cultures results from decreased synthesis of ribonucleic acid (137). It is entirely possible that the tissue effects of iron deficiency in higher organisms may be mediated through a similar mechanism.

Summary

Great progress was made in the understanding of iron metabolism between 1930 and 1950. However, it has been necessary to re-evaluate and

revise some of the coocepts formulated during this period of time. The means by which the iron traverses the mucosa is not well understood. Ferritin seems to be formed in mucosal cells when iron is fed, but it is not clear whether its function is storage or transport. The incorporation of iron into ferritin and the release of iron from ferritin has been studied both *in vitro* and *in vivo*. Some evidence has been presented that the xanthine oxidase system may play a role in release of iron from ferritin, but the physiological importance of this mechanism is not yet considered to be firmly established. The absorption of iron is increased regularly by iron deficiency, acute bleeding, cobalt, anoxia, and by hemolysis. It is decreased by hypertransfusion and possibly by aplastic anemia. The mechanism of control of iron absorption is not understood, but it is suggested that it may be dual in nature. The level of the iron stores of the organism seem to exert an influence, but another factor, related either to bone marrow activity or to bowel anoxia, also appears to play a role. Severe iron deficiency anemia can be distinguished reliably from other forms of anemia by means of clinical history, red cell morphology and, in obscure cases, by plasma iron and iron-binding capacity. Mild iron deficiency anemia can be detected most reliably on the basis of the examination of bone marrow for stainable iron. Plasma iron determination, iron-binding capacity determination, and possibly iron-tolerance curves may be of some value. Not only are storage iron, plasma transport iron, and hemoglobin affected in the iron deficiency state, but the activities or concentrations of certain vital iron enzymes are affected as well. Specifically, cytochrome-*c* and myoglobin concentrations and the activity of aconitase are reduced in the tissues of iron-deficient experimental animals. Cytochrome oxidase and succinic dehydrogenase are affected to much lesser degrees. Catalase appears to be resistant to depletion in iron-deficient organisms.

LITERATURE CITED

1. Bass, R. L., Bernick, S., and Saltman, P., *Exptl. Cell Research*, 13, 395–97 (1957)
2. Bass, R., and Saltman, P., *Exptl. Cell Research*, 18, 560–72 (1959)
3. Becquerel, A., and Rodier, A., *Gaz. med. Paris*, 12, 751 (1844)
4. Beifeld, A. F., *Med. Clin. Chicago*, 2, 963–89 (1917)
5. Beinert, H., and Sands, R. H., *Biochem. Biophys. Research Communs.*, 1, 171–74 (1959)
6. Bessis, M., and Breton-Gorius, J., *Rev. hématol.*, 12, 43–63 (1957)
7. Beutler, A., *Folia Haematol.*, 29, 121 (1923)
8. Beutler, E., *Blut*, 6, 130–33 (1960)
9. Beutler, E., *Acta Haematol.*, 21, 371–77 (1959)
10. Beutler, E., *Am. J. Med. Sci.*, 234, 517–27 (1957)
11. Beutler, E., *J. Clin. Invest.*, 38, 1605–16 (1959)
12. Beutler, E., *Nature*, 181, 837–38 (1958)
13. Beutler, E. (Unpublished observations)
14. Beutler, E., *J. Lab. Clin. Med.*, 51, 415–19 (1958)
15. Beutler, E., *New Engl. J. Med.*, 256, 692–97 (1957)
16. Beutler, E., *Ann. Internal Med.*, 50, 313–22 (1959)
17. Beutler, E., and Blaisdell, R. K., *J. Clin. Invest.*, 37, 833–35 (1958)
18. Beutler, E., and Blaisdell, R. K., *J. Lab. Clin. Med.*, 52, 694–99 (1958)
19. Beutler, E., and Blaisdell, R. K., *Blood*, 15, 30–35 (1960)
20. Beutler, E., and Buttenwieser, E., *J. Lab. Clin. Med.*, 55, 274–80 (1960)
21. Beutler, E., Drennan, W., and Block, M., *J. Lab. Clin. Med.*, 43, 427–39 (1954)
22. Beutler, E., Larsh, S. E., and Gurney, C. W., *Ann. Internal. Med.*, 52, 378–94 (1960)
23. Beutler, E., Larsh, S., and Tanzi, F., *Am. J. Med. Sci.*, 239, 759–65 (1960)
24. Beutler, E., Robson, M., and Buttenwieser, E., *Ann. Internal Med.*, 48, 60–82 (1958)
25. Bielig, H. J., and Bayer, E., *Naturwissenschaften*, 42, 466 (1955)
26. Bielig, H. J., and Bayer, E., *Naturwissenschaften*, 42, 125–26 (1955)
27. Boddington, M. M., *J. Clin. Pathol.*, 12, 222–27 (1959)
28. Bonnet, J. D., Hagedorn, A. B., and Owen, C. A., Jr., *Blood*, 15, 36–44 (1960)
29. Bonnet, J. D., Orvis, A. L., Hagedorn, A. B., and Owen, C. A., Jr., *Am. J. Physiol.*, 198, 784–86 (1960)
30. Bothwell, T. H., and Finch, C. A., *Am. J. Digest. Diseases*, 2, 145–58 (1957)
31. Bothwell, T. H., Pirzio-Biroli, G., and Finch, C. A., *J. Lab. Clin. Med.*, 51, 24–36 (1958)
32. Bothwell, T. H., Pribilla, W., and Hurtado, A., Jr., "The Role of Erythropoietic Activity in Regulating Iron Transport," 313, *Intern. Soc. Hematol., 6th Congr., Boston, 1956* (Grune & Stratton, Inc., 1958)
33. Brown, E. B., Jr., Dubach, R., and Moore, C. V., *J. Lab. Clin. Med.*, 52, 335–55 (1958)
34. Brown, E. B., Jr., and Justus, B. W., *Am. J. Physiol.*, 194, 319–26 (1958)
35. Brown, E. B., and Moore, C. V., *Progress in Hematology*, I, 24–46 (Grune & Stratton, Inc., New York, N. Y., 1956)
36. Brown, E. G., *Nature*, 182, 313–15 (1958)
37. Brumfitt, W., *Quart. J. Med.*, 29, 1–18 (1960)
38. Cartwright, G. E., Huguley, C. M., Ashenbrucker, H., Fay, J., and Wintrobe, M. M., *Blood*, 3, 501–25 (1948)
39. Cartwright, G. E., and Wintrobe, M. M., *J. Clin. Invest.*, 28, 86–98 (1949)
40. Cheney, B., and Finch, C. A., *Proc. Soc. Exptl. Biol. Med.*, 103, 37–38 (1960)
41. Cohen, E., and Elvehjem, C. A., *J. Biol. Chem.*, 107, 97–105 (1934)
42. Crawley, J., *Edinburgh Med. J.*, 59, 478–91 (1952)
43. Davidson, W. M., and Jennison, R. F., *J. Clin. Pathol.*, 5, 281–85 (1952)
44. Demulder, R., *Arch. Internal Med.*, 102, 254–301 (1958)
45. Dern, R. J., and Hart, W. L., *J. Lab. Clin. Med.*, (In press)
46. Dickman, S. R., and Cloutier, A. A., *J. Biol. Chem.*, 188, 379–88 (1951)
47. Douglas, A. S., and Dacie, J. V., *J. Clin. Pathol.*, 6, 307–13 (1953)
48. Dowdle, E. B., Schachter, D., and Schenker, H., *Am. J. Physiol.*, 198, 609–13 (1960)
49. Dubach, R., Callender, S. T. E., and Moore, C. V., *Blood*, 3, 526–40 (1948)
50. Dubach, R., Moore, C. V., and

Callender, S., *J. Lab. Clin. Med.*, 45, 599–615 (1955)
51. Editorial, *Lancet*, II, 27–28 (1960)
52. Editorial, *Brit. Med. J.*, I, 1167–68 (1958)
53. Field, M., Seki, M., Mitchell, M. L., and Chalmers, T. C., *J. Lab. Clin. Med.*, 55, 929–35 (1960)
54. Finch, C. A., *J. Clin. Invest.*, 38, 392–96 (1959)
55. Fridovich, I., and Handler, P., *J. Biol. Chem.*, 231, 899–911 (1958)
56. Gabrio, B. W., and Salomon, K., *Proc. Soc. Exptl. Biol. Med.*, 75, 124–27 (1950)
57. Gillmann, H., *Deut. med. Wochschr.*, 84, 1737–41 (1959)
58. Gillman, T., and Hathorn, M., *Brit. Med. J.*, II, 635–36 (1958)
59. Gisinger, E., *Wien. Z. inn. Med. u. Grenzg.*, 34, 395–400 (1953)
60. Goldeck, H., Remy, D., and Labhard, H., *Deut. med. Wochschr.*, 79, 211–13 (1954)
61. Goldeck, H., Remy, D., and Pang, P. K., *Deut. Arch. klin. Med.*, 199, 239–50 (1952)
62. Govan, A. D. T., and Scott, J. M., *Lancet*, I, 14–16 (1949)
63. Granick, S., *Physiol. Revs.*, 31, 489–511 (1951)
64. Granick, S., *J. Biol. Chem.*, 164, 737–46 (1946)
65. Granick, S., *Bull. N. Y. Acad. Med.*, 25, 403–28 (1949)
66. Granick, S., and Michaelis, L., *J. Biol. Chem.*, 147, 91–97 (1943)
67. Green, S., and Mazur, A., *J. Biol. Chem.*, 227, 653–68 (1957)
68. Gubler, C. J., *Science*, 123, 87–90 (1956)
69. Gubler, C. F., Cartwright, G. E., and Wintrobe, M. M., *J. Biol. Chem.*, 224, 533–46 (1957)
70. Hahn, P. F., *Medicine*, 16, 249–66 (1937)
71. Hahn, P. F., *Federation Proc.*, 7, 493–98 (1948)
72. Hahn, P. F., Bale, W. F., Ross, J. F., Balfour, W. M., and Whipple, G. H., *J. Exptl. Med.*, 78, 169–88 (1943)
73. Hahn, P. F., and Whipple, G. H., *Am. J. Med. Sci.*, 191, 24–42 (1936)
74. Hansen, H. A., and Weinfeld, A., *Acta Med. Scand.*, 165, 333–56 (1956)
75. Heath, C. W., and Patek, A. J., *Medicine*, 16, 267–350 (1937)
76. Heilmeyer, L., and Plötner, K., *Das Serumeisen und die Eisenmangelkrankheit. Pathogenese, Symptomatologie und Therapie* (Gustav Fischer, Verlag, Jena, Germany 1937)
77. Heistø, H., and Foss, P. O., *Scand. J. Clin. & Lab. Invest.*, 10, 102–7 (1958)
78. Holly, R. G., *Obstet. and Gynecol.*, 2, 119–26 (1953)
79. Hussian, R., and Patwardhan, V. N., *Lancet*, I, 1073–74 (1959)
80. Hutchison, H. E., *Blood*, 8, 236–48 (1953)
81. Jacobs, A., *J. Clin. Pathol.*, 12, 234–37 (1959)
82. Jandl, J. H., Inman, J. K., Simmons, R. L., and Allen, D. W., *J. Clin. Invest.*, 38, 161–85 (1959)
83. Jasinski, B., *Acta Haematol.*, 3, 17–26 (1950)
84. Jasinski, B., *Schweiz. med. Woehschr.*, 79, 1255–58 (1949)
85. Jasinski, B., *Schweiz. med. Woehschr.*, 80, 59–62 (1950)
86. Jasinski, B., and Roth, O., *Larvierte Eisenmangelkrankheit*, (Benno, Schwabe, Basel, Switzerland, 1954)
87. Josephs, H. W., *Blood*, 13, 1–54 (1958)
88. Kampschmidt, R. G., Adams, M. E., and Goodwin, W. L., *Arch. Biochem. Biophys.*, 82, 42–49 (1959)
89. Korman, S., *Federation Proc.*, 18, 578 (1959)
90. Korman, S., and Laszlo, D., *Federation Proc.*, 17, 257 (1958)
91. Krantz, S., Goldwasser, E., and Jacobson, L. O., *Blood*, 14, 654–61 (1959)
92. Laache, S., *Die Anämie* (Walling, Oslo, Norway, 1883)
93. Lees, F., and Rosenthal, F. D., *Quart. J. Med.*, 27, 19–26 (1958)
94. Lentino, W., Collica, C., and Rubenfeld, S., *J. Am. Med. Assoc.*, 173, 481–87 (1960)
95. Leonard, B. J., *Lancet*, I, 899–902 (1954)
96. Ley, A. B., *J. Clin. Invest.*, 39, 1006 (1960)
97. Loewus, M. W., and Fineberg, R. A., *Biochim. et Biophys. Acta*, 26, 441–43 (1957)
98. Loftfield, R. B., and Eigner, E. A., *J. Biol. Chem.*, 231, 925–43 (1958)
99. Mazur, A., Green, S., and Carleton, A., *J. Biol. Chem.*, 235, 595–603 (1960)
100. Mazur, A., Green, S., Saha, A., and Carleton, A., *J. Clin. Invest.*, 37, 1809–17 (1958)
101. McCance, R. A., and Widdowson, E. M., *J. Physiol.*, 94, 148–54 (1938)

102. Moore, C. V., *Am. J. Clin. Nutrition*, 3, 3–10 (1955)
103. Moore, C. V., *Scand. J. Clin. & Lab. Invest.*, 9, 292–304 (1957)
104. Morawitz, P., *Münch. med. Wochschr.*, 57, 1425–30 (1910)
105. Naegeli, O., *Blutkrankheiten und Blutdiagnostik Lehrbuch der morphologischen Hämatologie*, 232, 237 (Verlag Von Veit, Leipzig, Germany, 1908)
106. Patwardhan, M. V., *Nature*, 181, 187 (1958)
107. Pirzio-Biroli, G., Bothwell, T. H., and Finch, C. A., *J. Lab. Clin. Med.*, 51, 37–48 (1958)
108. Pirzio-Biroli, G., and Finch, C. A., *J. Lab. Clin. Med.*, 55, 216–20 (1960)
109. Pratt, P. T., and Johnson, M. E., *Arch. Internal Med.*, 93, 725–30 (1954)
110. Rath, C. E., and Finch, C. A., *J. Lab., Clin. Med.*, 33, 81–86 (1948)
111. Rath, C. E., and Finch, C. A., *J. Clin. Invest.*, 28, 79–85 (1949)
112. Roth, O., Jasinski, B., and von Bidder, H., *Helv. Med. Acta*, 18, 159–74 (1951)
113. Rummel, W., and Candon, B., *Intern. Record Med. & Gen. Pract. Clins.*, 169, 783–84 (1956)
114. Rustang, E., *Acta Dermato-Venereol.*, 29, Suppl. 21, 1–140 (1949)
115. Sahli, H., *Lehrbuch der Klinischen Untersuchungs – Methoden*, 993 (Franz Deuticke, Leipzig & Wien, 1908)
116. Saltman, P., Fiskin, R. D., and Bellinger, S. B., *J. Biol. Chem.*, 220, 741–50 (1956)
117. Saltman, P., Fiskin, R. D., Bellinger, S. B., and Alex, T., *J. Biol. Chem.*, 220, 751–57 (1956)
118. Saylor, L., and Finch, C. A., *Am. J. Physiol.*, 172, 372–76 (1953)
119. Smith, M. D., and Pannacciulli, I. M., *Brit. J. Haematol.*, 4, 428–34 (1958)
120. Sturgeon, P., *Brit. J. Haematol.*, 5, 31–44 (1959)
121. Sturgeon, P., Itano, H. A., and Bergren, W. R., *Brit. J. Haematol.*, 1, 264–77 (1955)
122. Stevens, A. R., *Arch. Internal. Med.*, 98, 550–54 (1956)
123. Stevens, A. R., Coleman, D. H., and Finch, C. A., *Ann. Internal Med.*, 38, 199–205 (1953)
124. Stewart, W. B., Vassar, P. S., and Stone, R. S., *J. Clin. Invest.*, 32, 1225–28 (1953)
125. Sturgis, C. C., *Hematology*, 2nd ed., 78 (Charles C Thomas, Publ., Springfield, Ill., 1957)
126. Suda, M., and Takeda, Y., *J. Biochem.* 37, 381–85 (1950)
127. Tanaka, S., *J. Biochem.*, 37, 129–43 (1950)
128. Thedering, F., *Med. Klin. (Munich)*, 50, 1463–67 (1955)
129. Thedering, F., *Medizinische*, 38, 1224–28 (1953)
130. Vannotti, A., *Schweiz. med. Wochschr.*, 79, 261–63 (1949)
131. Verloop, M. C., Meeuwissen, J. E. T., Blokhuis, E. W. M., *Brit. J. Haematol.*, 4, 70–81 (1958)
132. Vries, A., and Izak, G., *Rev. hématol.*, 10, 657–64 (1955)
133. Waldenström, J., *Acta Med. Scand., Suppl. 170*, 252–79 (1946)
134. Wallerstein, R. O., *J. Am. Med. Woman's Assoc.*, 9, 149–50 (1954)
135. Wallerstein, R. O., and Aggeler, P. M., *California Med.*, 84, 176–79 (1956)
136. Wenderoth, H., *Acta Haematol.*, 5, 338–43 (1951)
137. Winder, F., and Denneny, J. M., *Nature*, 184, 742–43 (1959)
138. Wöhler, F., Heilmeyer, L., Emrich, D., and Kang, S. H., *Arch. exptl. Pathol., Pharmakol., Naunyn-Schmiedeberg's*, 230, 107–24 (1957)
139. Yu, C. F., *Chinese Med. J.*, 77, 347–55 (1958)

BILE PIGMENT METABOLISM[1,2]

By Gerald Klatskin, M. D.[3]

Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut

The bile pigments, a group of pyrrolic compounds, constitute the end products of the catabolism of hemoglobin and possibly other related tetrapyrroles. Arising in the reticuloendothelial system, they enter the plasma to be excreted by the liver, ultimately leaving the body in the feces and, to a lesser extent, in the urine. Under a variety of conditions leading to increased production or faulty excretion, the pigments accumulate and stain the tissues giving rise to jaundice. Although the structure and function of most tissues are not affected by such staining, serious injury in the form of kernicterus may ensue when the pigments gain access to the brain during the neonatal period.

Great strides have been made in elucidating the metabolic pathways of the bile pigments and the mechanisms underlying the development of jaundice. However, as will be evident from the following review, there are still a number of important gaps in our knowledge.

NORMAL BILE PIGMENT METABOLISM

Degradation of hemoglobin to bilirubin.—Based on measurements of the survival in the circulation of newly-formed erythrocytes containing labeled hemoglobin following the administration of N^{15} glycine, it has been estimated that the life span of the normal red cell is approximately 120 days (1). On reaching senescence, the red cell is taken up by the reticuloendothelial system and destroyed, the end products being bilirubin, iron, and globin (2). The bilirubin is discharged into the plasma, ultimately to be excreted by the liver. In contrast, the iron is conserved for further use, some being stored locally in the form of hemosiderin, a ferric hydroxide polymer, while the remainder is returned to the plasma where, in combination with the *beta*$_1$-globulin, siderophilin, it is transported to other tissues for storage as hemosiderin or as the ferrous iron-protein complex, ferritin (3). Little is known about the fate of globin, although, on the basis of indirect evidence, it has been suggested that it enters a labile protein pool and can be reutilized in hemoglobin synthesis (4). The view held by some (5) that globin remains at-

[1] The survey of the literature pertaining to this review was concluded in June, 1960.

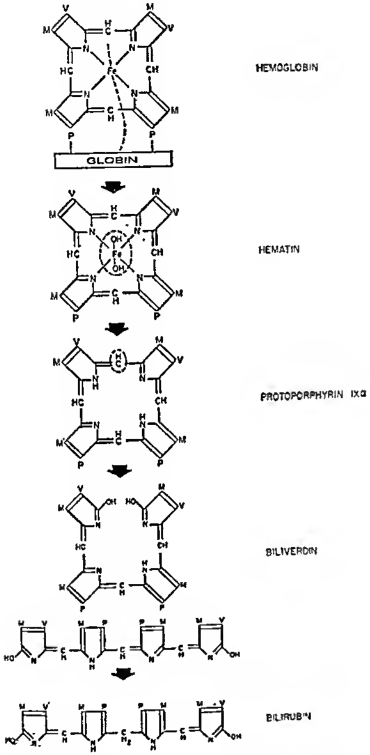
[2] The following abbreviations will be used: ATP (adenosine triphosphate); DPN, DPNH (diphosphopyridine nucleotide and reduced form); PP (inorganic pyrophosphate); UDP (uridine diphosphate); UDPG (uridine diphosphate glucose); UDPGA (uridine diphosphate glucuronic acid); UTP (uridine triphosphate).

[3] Supported by a research grant (A-714) from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, U. S. Public Health Service.

tached to the prosthetic group of hemoglobin during the degradation of heme and leaves the reticuloendothelium firmly bound to bilirubin to be separated from it in the liver, is not supported by recent electrophoretic studies (6). These have shown that the protein with which bilirubin is associated in the serum is not globin (*vide infra*). It is highly probable, therefore, that globin splits off within the reticuloendothelial system at some stage during the degradation of hemoglobin. The subsequent pathway of globin metabolism is uncertain. Although free globin has not been identified in serum (6), the methods employed have been relatively insensitive, so that the possibility cannot be excluded that globin leaves the reticuloendothelium in its native state to be metabolized elsewhere.

The precise sequence of events involved in the degradation of hemoglobin to bilirubin is uncertain. According to some authorities (7), the first step involves splitting of the molecule to yield globin and hematin, an hydroxide of the trivalent-iron derivative of heme. The latter is then converted to protoporphyrin IXα by the removal of iron, following which it undergoes oxidation at its α-methene bridge with loss of a carbon atom. As a result, the porphyrin ring opens to form a straight-chain tetrapyrrole, biliverdin which, on reduction of its central methene bond, yields bilirubin (Fig. 1). That hematin can give rise to bilirubin has been established in both *in vitro* (8) and *in vivo* (9) experiments, and undoubtedly accounts for the increase in bilirubin production that occurs under pathological conditions known to be accompanied by hematin formation such as in intravascular hemolysis (10), some forms of advanced hepatocellular disease (11), and extravasation of blood into the tissues or body cavities (2). However, there is no clear-cut evidence that hematin is an intermediate in the catabolism of hemoglobin released from senescent erythrocytes undergoing destruction in the reticuloendothelium, although the small fraction of bilirubin derived from sources other than the hemoglobin of circulating erythrocytes may stem from hematin produced in the bone marrow (12, 13). Attempts to demonstrate the conversion of protoporphyrin to bile pigment *in vivo* by the injection of protoporphyrin (14), or *in vitro* by the coupled oxidation of protoporphyrin with ascorbic acid, a method that yields bile pigments with hematin, hemoglobin, and a variety of other heme derivatives (8), have met with failure, suggesting that the presence of iron within the porphyrin molecule may be essential for cleavage of its ring structure. Recently, London and his associates (15) have reported that several days following the injection of C^{14}-labeled protoporphyrin it was possible to demonstrate a small fraction of the carbon label in the fecal bile pigments. However, as pointed out by Lemberg (16), the possibility was not excluded that iron was introduced into the protoporphyrin molecule before its conversion to bilirubin. Although not conclusive, the evidence available at the present time supports the view that protoporphyrin is not an intermediate in the production of bile pigments from either hemoglobin or hematin.

An alternative pathway for the degradation of hemoglobin that is more



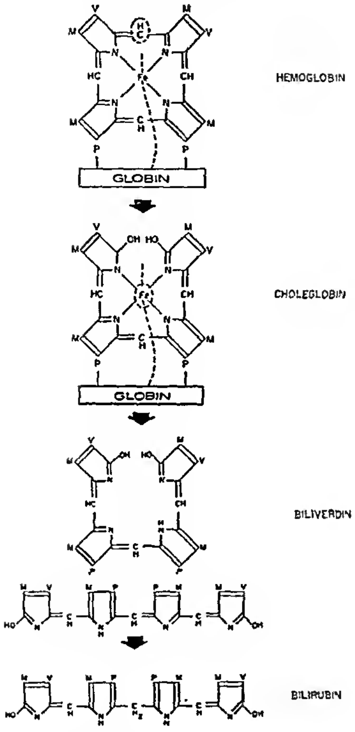
M = $-CH_3$ (Methyl)
V = $-CH=CH_2$ (Vinyl)
P = $-CH_2-CH_2-COOH$ (Proprionic Acid)

Fig. 1. Degradation of hemoglobin to bilirubin via the hematin pathway.

widely accepted has been proposed by Lemberg and his associates (16, 17). According to these investigators, the initial step involves the oxidative removal of the carbon atom in the α-methene bridge which opens the porphyrin ring to yield choleglobin, a green, iron-containing, pigment-protein complex. By subsequent removal of iron and globin, the choleglobin is converted to biliverdin which, as previously described, is reduced to bilirubin (Fig. 2). It has not been possible to isolate choleglobin in pure form, so that its precise structure is not known. The formula shown in Figure 2 is one of several that have been suggested. There is a possibility that an oxygen bond replaces the α-methene bridge in the initial oxidation of the porphyrin ring, and that it is removed subsequently to yield the structure illustrated. Lemberg (16) suggests that the conversion of hemoglobin to choleglobin probably involves its coupled oxidation with the ascorbic acid-glutathione system, since this reaction can be demonstrated *in vitro* under physiological conditions of temperature, pH, oxygen tension, and hydrogen donor concentration. He points out that, in contrast, the *in vitro* conversion of hematin or of its albumin complex, methemalbumin, requires an ascorbic acid concentration and oxygen tension that are never attained in the tissues. As further support for his hypothesis, Lemberg cites the fact that he and his associates have demonstrated the presence of choleglobin and biliverdin in normal rabbit erythrocytes, and that the amounts of these substances increase substantially when animals are poisoned with phenylhydrazine, an agent known to induce *in vivo* hemolysis. This suggests that the degradation of hemoglobin may begin in intact senescent erythrocytes before they are engulfed and destroyed in the reticuloendothelial system. However, Gardikas and his associates (18) have not been able to confirm these observations in human erythrocytes, so that the importance of intracorpuscular degradation of hemoglobin is in doubt.

From the relative molecular weights of hemoglobin (68,000) and bilirubin (572), and the fact that one mole of the former contains four of the latter, it may be calculated that on degradation one gram of hemoglobin yields approximately 34 mg. of bilirubin. Assuming that the average adult has a blood volume of approximately 5 l. and a hemoglobin concentration of 15 gm. per 100 ml., and destroys almost 1 per cent of the circulating erythrocytes daily as a consequence of senescence, it may be estimated that normally 7.5 gm. of hemoglobin are released for degradation resulting in a daily production of approximately 250 mg. of bilirubin. Recently, Crosby (19), by infusing increasing amounts of hemoglobin into healthy subjects and measuring the levels of serum bilirubin and hemoglobin attained, has demonstrated that the maximum capacity of the reticuloendothelial system to convert hemoglobin to bilirubin lies between 45 and 50 gm. of hemoglobin per day, equivalent to a daily output of approximately 1.5 gm. of bilirubin.

Sources of bilirubin other than the hemoglobin of circulating erythrocytes.— Recent studies indicate that normally from 10 (12) to 30 (13) per cent of the bilirubin formed is derived from sources other than the hemoglobin of circulating erythrocytes that have reached the end of their life span, and that



M = $-CH_3$ (Methyl)
V = $-CH=CH_2$ (Vinyl)
P = $-CH_2-CH_2-COOH$ (Propionic Acid)

FIG. 2. Degradation of hemoglobin to bilirubin via the choleglobin pathway.

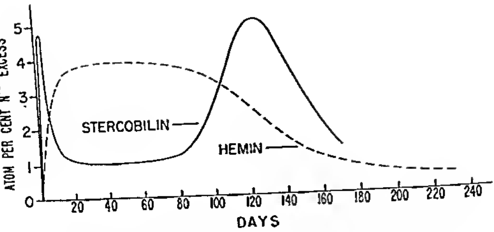


FIG. 3. N^{15} content of circulating hemin and fecal stercobilin following the administration of N^{15} Glycine [modified after London (12) and Gray (13)].

under pathological conditions such as pernicious anemia (20) and congenital porphyria (13, 21), this fraction may constitute an even greater proportion of the total output of bile pigment. Thus, London (12) and Gray (13) and their associates found that, when N^{15} glycine was administered to normal subjects for two to four days, N^{15} was demonstrable in the bile pigment (stercobilin) isolated from the feces before any of the newly-formed, circulating erythrocytes containing N^{15}-labeled hemoglobin had been destroyed. In Figure 3, which depicts the results in one of these experiments, it will be observed that the curve for the concentration of N^{15} stercobilin showed a peak immediately following the administration of labeled glycine (Fraction I), fell to a low plateau that was maintained between day 20 and day 80 (Fraction II), and then rose to a second peak that began at about 80 days and was maximum at 130 days (Fraction III). Since the initial peak occurred at a time when the N^{15} concentration in the hemin isolated from circulating erythrocytes was just beginning to rise, i.e., as newly-formed cells containing the label were starting to enter the circulation, and when only unlabeled cells produced 120 days earlier were being destroyed, it is apparent that Fraction I of stercobilin could not have been derived from the hemoglobin of circulating erythrocytes. Similarly, the latter could not bave been the source of Fraction II since, as demonstrated in Figure 3, the concentration of N^{15} in heme during this period was remarkably stable, indicating that none of the labeled erythrocytes were being destroyed. In contrast, the N^{15} stercobilin peak observed between the 80th and 130th days coincided with the fall in the concentration of N^{15} hemin caused by the destruction of labeled erythrocytes that had reached the end of their normal life span. Accordingly, it was reasonably certain that Fraction III of stercobilin was derived from the hemoglobin of circulating erythrocytes. Several possible sources for Fraction I of stercobilin have been suggested: (a) heme formed in excess of globin during the synthesis

of hemoglobin; (*b*) intracorpuscular degradation of hemoglobin during the maturation of the early erythrocyte in the bone marrow; (*c*) destruction of newly-formed erythrocytes in the bone marrow before they reach the circulation; (*d*) direct synthesis from a metabolic pool of pyrroles; and (*e*) other heme proteins such as myoglobin, catalase, and the cytochromes (12, 13). Of these the first three appear most likely. With respect to Fraction II of stercobilin, Gray (22) favors the view that it is derived from myoglobin and other non-hemoglobin heme proteins. In this connection it is noteworthy that Kench (8) has demonstrated the *in vitro* formation of bile pigments on coupled oxidation of ascorbic acid with myoglobin, catalase, and peroxidase, but not with cytochrome-*c*.

Transport of bilirubin in plasma.—Bilirubin is virtually insoluble in water at the pH of the blood (23). However, it has a great avidity for serum albumin, forming a relatively stable complex with the latter in a ratio of between two and three moles to one (24), a state in which it is transported in the plasma (6). Other proteins are capable of binding bilirubin (24) but in the plasma the latter appears to combine with albumin preferentially (6), although on electrophoretic analysis it is possible to demonstrate a small but constant amount of bilirubin in the α-globulin fraction (25). On separating the plasma proteins by the Cohn method which entails fractionation in alcohol-water mixtures at subzero temperatures under varying conditions of pH, salt, and protein concentration, the only bilirubin-protein complex to survive this treatment is found in the α_1-globulin of Fraction V-1 (26). Because of its stability, Cohn (26) regarded it as the only real bile pigment-protein in plasma. However, its concentration is so low that it cannot account for more than 0.05 mg. of bound bilirubin per 100 ml.

Normally, the linkage between albumin and bilirubin is sufficiently stable to prevent the passage of bilirubin through a semipermeable membrane (27). However, certain organic anions used as drugs, such as salicylate and sulfisoxazole, at concentrations achieved therapeutically, are capable of uncoupling the complex and rendering the bilirubin ultrafiltrable (28). Similarly, lowering the pH of the serum to below 5.0, as in the van den Bergh reaction, results in separation of bilirubin from albumin (6).

The level of bilirubin in the plasma is determined by the relative rates at which bilirubin enters and leaves the circulation (29). Normally the concentration lies between 0.5 and 1.0 mg. per 100 ml. The precise upper limit of normality is difficult to define, since the distribution curve of values obtained from allegedly healthy adults is asymmetrical and skewed to the right (30), suggesting that a significant number of individuals in a presumably normal population may have unrecognized abnormalities of pigment metabolism (*vide infra*). Based on a statistical analysis of a very large population, the upper limit of normal has been designated as 1.5 mg. per 100 ml. (30). In other species, such as the dog, whose capacity for excreting bilirubin is greater than that of man, the plasma is virtually devoid of bilirubin (31).

Uptake and excretion of bilirubin by the liver.—Bilirubin is removed from

the plasma and excreted in the bile by a dynamic process that appears to involve several distinct but interdependent steps. That the uptake and excretion of bilirubin represent two such distinct steps is evident from the observation that following an injection of bilirubin, pigment is cleared from the plasma earlier and at a more rapid rate than it is excreted in the bile (31, 32). The period of delay between uptake and excretion, during which bilirubin is stored in the liver, appears to depend, in part at least, on the important intermediate step of bilirubin conjugation (*vide infra*) since, if instead of free bilirubin its glucuronide is injected, the pigment is cleared from the plasma and recovered in the bile without delay (32). Conjugation also affects pigment uptake and excretion. Thus, in the Gunn rat (33), a mutant strain of Wistar rat with congenital jaundice caused by a hereditary deficiency of the principal conjugating enzyme within the liver, the clearance from the plasma of injected unconjugated bilirubin is greatly delayed and virtually none is recovered in the bile, while both the uptake and excretion of conjugated bilirubin are normal (32, 34). Although conjugation appears to be a critical intermediate step that may limit either the uptake or excretion of bilirubin, there are several clinical situations in which the excretion is abnormal despite a normal conjugating system and patent biliary tree. This suggests that an active transport system may be required for the movement of bilirubin from the cell membrane facing the sinusoids, to the microsomes for conjugation, and then to the cell membrane facing the canaliculi for excretion, and that disturbances involving this mechanism may be responsible for some forms of jaundice. Support for this view is to be found in the work of Hanzon (35) based on direct microscopic studies of the excretion of uranin, a fluorescent dye that behaves like bilirubin. From these observations, Hanzon has concluded that bilirubin diffuses passively through the sinusoidal endothelial cells, is concentrated close to the endothelial surface of the liver cell by an active process, diffuses passively across the cell as a consequence of the concentration gradient developed, is then actively concentrated a second time close to the canalicular surface, ultimately to be excreted into the bile. He has stressed the unidirectional character of this process, and has demonstrated that hepatocellular injury or biliary obstruction impairs the two active concentrating processes within the cell, and ultimately affects the permeability of the cell membrane so that the polarity of the cell is reversed, permitting the passage of bilirubin from the canaliculus to the sinusoid.

Weech and his associates (29) have shown that, within certain limits, the amount of bilirubin excreted by the normal liver is proportional to the square of its concentration in the plasma. This accounts for the observation (29) that, when the rate at which bilirubin enters the circulation is increased, the plasma concentration rises but soon stabilizes at a new level; under these conditions the rate of excretion also increases, ultimately to equal that of bilirubin entering the plasma, and thus establishes a new state of equilibrium. This is an important point in connection with the level of plasma bilirubin to be expected in hemolytic jaundice (*vide infra*). By direct measurement of

bilirubin in the bile following its injection intravenously, it can be shown that there is a limit to the excretory capacity of the liver, and that when this is exceeded there is a progressive rise in the serum bilirubin level. In the rat, Weinbren & Billing (36) found that the maximum excretory rate was 61 ± 8.0 µg./min./100 gm. body weight, a level reached when the serum concentration exceeded 15 mg./100 ml. Lathe & Walker (37) confirmed these observations and pointed out that, making allowances for injury in the preparation of liver suspensions, there was remarkable agreement in their experiment between the rate of bilirubin conjugation in such suspensions (0.4 mg./gm. wet weight/hr.) and the maximum rate of bilirubin excretion in the intact animal (1.2 mg./gm. wet weight of liver/hr.). This suggests that in the normal liver the capacity of the enzymatic conjugating system may be the limiting factor that determines the maximum rate of bilirubin excretion.

Bilirubin conjugation and the van den Bergh reaction.—The characteristic coupling reaction of bilirubin with diazotized sulfanilic acid, now known to involve splitting of the bilirubin molecule to form two relatively stable dipyrryl azopigment molecules (38), was first described by Ehrlich in 1883 (39). Since Ehrlich used chloroform as a solvent for bilirubin, he found it necessary to add alcohol to permit solution of the otherwise immiscible aqueous reagent. Fifty years later, van den Bergh & Snapper (40) applied this reaction to the quantitative estimation of bilirubin in body fluids, and in doing so continued the practice of including alcohol in the reaction mixture. However, in 1916 van den Bergh & Müller (41) discovered, quite by accident, that alcohol could be omitted in carrying out the reaction in bile and in icteric sera from patients with obstructive jaundice, but not in aqueous alkaline solutions of crystalline bilirubin or in icteric sera from patients with hemolytic jaundice. To these two types of reaction they applied the terms "direct" and "indirect," respectively, indicating that the former required no alcohol while the latter did. In the four decades that ensued before the problem was solved, numerous theories were advanced to account for the variable behavior of bilirubin in the van den Bergh reaction. Of these, the two that survived the longest were (*a*) that the two types of reactions are dependent upon differences in the linkage of bilirubin to protein, and (*b*) that bilirubin occurs in two structurally distinct forms, only one of which is capable of coupling with diazonium salts in the absence of alcohol.

Conclusive proof that the two reactions are not dependent upon differences in protein linkage was provided by Cole & Lathe (42) in 1953. These investigators succeeded in separating protein-free preparations of direct- and indirect-reacting bilirubin by reverse phase chromatography on silicone-treated kieselguhr, using a solvent system of chloroform, methanol, carbon tetrachloride, and phosphate buffer at pH 6. The indirect-reacting pigment, which was soluble in organic solvents and moved down the column slowly, was shown to be identical with crystalline bilirubin, while the direct-reacting pigment which migrated more rapidly proved to be water-soluble at an acid pH. In a subsequent study (43), these workers, in collaboration with Billing,

two molecules of azopigment on diazotization, these investigators concluded that Pigment II, which gives rise to azopigment B exclusively, is a diglucuronide of bilirubin, and that Pigment I, which yields both azopigment A and B, is a monoglucuronide. They suggested, moreover, that an ester linkage between glucuronic acid and the proprionic acid side chains was more likely than a glycosidic linkage through the hydroxyl groups of bilirubin, in view of the ease with which the conjugates were converted to free bilirubin by mild alkali treatment. Subsequently, Schachter (52) confirmed this impression by demonstrating that the direct-reacting pigment in the urine of patients with obstructive jaundice and the azopigment B derived from it yield hydroxamic acids on treatment with hydroxyalamine, a reaction characteristic of carboxyl glucuronides. The structural relationships between bilirubin and its glucuronides and their respective azopigment derivatives are shown graphically in Figure 4.

Schmid (49, 53) isolated azopigments A and B from diazotized samples of icteric serum, urine, and bile by ascending paper chromatography, using a solvent system of ethyl methyl ketone, *n*-proprionic acid, and water. Since, on acid hydrolysis or incubation with β-glucuronidase, azopigment B yielded equimolar amounts of azopigment A and glucuronic acid, while azopigment A underwent no change, he reached the same conclusion regarding the structure of the direct-reacting bile pigments as had Billing and her associates (51). Talafant (50) also suggested that the latter are glucuronide conjugates of bilirubin, since he found that on paper electrophoresis of partially purified dog bile, the direct-reacting pigment fraction was constantly associated with glucuronic acid in a molar ratio of approximately 1:2.

Billing and her associates (51) found that from 10 to 15 per cent of the direct-reacting pigment in human bile was alkali-stable, suggesting that this fraction was not a glucuronide of bilirubin. Recently, Isselbacher & McCarthy (54) have confirmed this observation and shown chromatographically that approximately 24 per cent of the total azopigment derivatives of the bilirubin in human bile resist hydrolysis by β-glucuronidase, and that approximately 14 per cent is in the form of a sulfate. They suggest that the remaining 9 to 10 per cent of the alkali-labile pigment that contains neither glucuronide nor sulfate may be a carboxyl-linked methyl or glycine conjugate of bilirubin. Since the alkali-stable pigment isolated by these investigators possessed a free carboxyl group, as evidenced by its failure to react with hydroxalamine, and yielded a methyl derivative on treatment with diazo methane, they concluded that the sulfate conjugate is linked with one or both hydroxyl groups of bilirubin.

The rate at which bilirubin diazotizes appears to be dependent upon its solubility. Undoubtedly, this accounts for the prompt direct reaction given by its conjugates which are freely soluble in water at the pH of the van den Bergh reaction. In contrast, unconjugated bilirubin is only negligibly soluble in water at this pH (23), and thus reacts so slowly that color development is weak and greatly delayed visually, although it is readily detected spectro-

demonstrated that by using a solvent system of n-butanol and phosphate buffer at pH 6, the latter could be furtber partitioned to yield two fractions, designated Pigments I and II, both of wbicb gave the direct van den Bergh reaction. On analysis the spectral absorption curves of the three types of bilirubin were similar, but could be distinguished from one another. In contrast, the absorption curves of tbeir respective azopigment derivatives were identical. Extracts of bile yielded Pigment II predominantly with lesser amounts of Pigment I aod traces of bilirubin. In bile-containing urine the pattern was similar, but Pigment I was the principal component. The serum bile pigment in hemolytic jaundice proved to be bilirubin, while that in obstructive jaundice consisted of a mixture of Pigments I and II. On the basis of a subsequent study of the serum pigments of jaundice, using a quantitative method based on the Cole-Lathe chromatographic technique (44), Billing (45) concluded that Pigment I is an intermediate in the formation of Pigmeot II from indirect-reacting bilirubin.

It was obvious from the similiarities of their spectral absorption curves and the fact that Pigments I and II could be converted to bilirubin (43) that the three pigments were closely related. However, the instability of Pigments I and II proved to be a stumbling block in establishing the nature of the structural differences that distinguished them. The discovery that on diazotization they yielded different azopigments that were stable and could be separated chromatographically proved to be an important step in the solution of this problem. Using a column of silicone-treated kieselguhr and a solvent system of n-butanol, water, and citrate buffer at pH 4, Billing (46) showed that the diazotization product of crystalline bilirubin (azopigment A) is relatively insoluble in water and, hence, moves slowly; in contrast that of Pigment II (azopigment B) is water-soluble and moves rapidly, while that of Pigment I behaves as a mixture of azopigments A and B. Subsequently, she pointed out that, because of the asymmetrical arrangement of the methyl and vinyl side chains in the bile pigments, each of the azopigments is made up of a mixture of two isomers differing only in the position of these side chains, and that although the isomers can be separated chromatographically their behavior is so similiar that they may be considered together as if they were one substance (47).

In 1956, by a remarkable coincidence, three groups of investigators working independently in England (48), io the United States (49), and in Czechoslovakia (50) simultaneously established that the direct-reacting bile pigment is a glucuronide of bilirubio. Billing, Cole & Lathe (48, 51) isolated a relatively pure preparation of azopigment B from diazotized human bile by the countercurrent distribution technique, and showed that on treatment with alkali or on enzymatic hydrolysis with β-glucuronidase it was converted to azopigment A with the release of a reducing substance which was identified chromatographically as glucurooic acid. By direct measurement, it was found that the latter was present in the original azopigment B in a molar ratio of 1:1, indicating a moooglucuronide structure. Since bilirubio yields

two molecules of azopigment on diazotization, these investigators concluded that Pigment II, which gives rise to azopigment B exclusively, is a diglucuronide of bilirubin, and that Pigment I, which yields both azopigment A and B, is a monoglucuronide. They suggested, moreover, that an ester linkage between glucuronic acid and the proprionic acid side chains was more likely than a glycosidic linkage through the hydroxyl groups of bilirubin, in view of the ease with which the conjugates were converted to free bilirubin by mild alkali treatment. Subsequently, Schachter (52) confirmed this impression by demonstrating that the direct-reacting pigment in the urine of patients with obstructive jaundice and the azopigment B derived from it yield hydroxamic acids on treatment with hydroxyalamine, a reaction characteristic of carboxyl glucuronides. The structural relationships between bilirubin and its glucuronides and their respective azopigment derivatives are shown graphically in Figure 4.

Schmid (49, 53) isolated azopigments A and B from diazotized samples of icteric serum, urine, and bile by ascending paper chromatography, using a solvent system of ethyl methyl ketone, *n*-proprionic acid, and water. Since, on acid hydrolysis or incubation with β-glucuronidase, azopigment B yielded equimolar amounts of azopigment A and glucuronic acid, while azopigment A underwent no change, he reached the same conclusion regarding the structure of the direct-reacting bile pigments as had Billing and her associates (51). Talafant (50) also suggested that the latter are glucuronide conjugates of bilirubin, since he found that on paper electrophoresis of partially purified dog bile, the direct-reacting pigment fraction was constantly associated with glucuronic acid in a molar ratio of approximately 1:2.

Billing and her associates (51) found that from 10 to 15 per cent of the direct-reacting pigment in human bile was alkali-stable, suggesting that this fraction was not a glucuronide of bilirubin. Recently, Isselbacher & McCarthy (54) have confirmed this observation and shown chromatographically that approximately 24 per cent of the total azopigment derivatives of the bilirubin in human bile resist hydrolysis by β-glucuronidase, and that approximately 14 per cent is in the form of a sulfate. They suggest that the remaining 9 to 10 per cent of the alkali-labile pigment that contains neither glucuronide nor sulfate may be a carboxyl-linked methyl or glycine conjugate of bilirubin. Since the alkali-stable pigment isolated by these investigators possessed a free carboxyl group, as evidenced by its failure to react with hydroxalamine, and yielded a methyl derivative on treatment with diazo methane, they concluded that the sulfate conjugate is linked with one or both hydroxyl groups of bilirubin.

The rate at which bilirubin diazotizes appears to be dependent upon its solubility. Undoubtedly, this accounts for the prompt direct reaction given by its conjugates which are freely soluble in water at the pH of the van den Bergh reaction. In contrast, unconjugated bilirubin is only negligibly soluble in water at this pH (23), and thus reacts so slowly that color development is weak and greatly delayed visually, although it is readily detected spectro-

demonstrated that by using a solvent system of n-butanol and phosphate buffer at pH 6, the latter could be further partitioned to yield two fractions, designated Pigments I and II, both of whicb gave the direct van den Bergh reaction. On analysis the spectral absorption curves of the three types of bilirubin were similar, but could be distinguished from one another. In contrast, the absorption curves of tbeir respective azopigment derivatives were identical. Extracts of bile yielded Pigment II predominantly with lesser amounts of Pigment I and traces of bilirubin. In bile-containing urine the pattern was similar, but Pigment I was the principal component. The serum bile pigment in hemolytic jaundice proved to be bilirubin, while that in obstructive jaundice consisted of a mixture of Pigments I and II. On the basis of a subsequent study of the serum pigments of jaundice, using a quantitative method based on the Cole-Lathe chromatographic tecbnique (44), Billing (45) concluded that Pigment I is an intermediate in the formation of Pigment II from indirect-reacting bilirubin.

It was obvious from the similiarities of their spectral absorption curves and the fact that Pigments I and II could be converted to bilirubin (43) that the three pigments were closely related. However, the instability of Pigments I and II proved to be a stumbling block in establishing the nature of the structural differences that distinguished them. The discovery that on diazotization they yielded different azopigments that were stable and could be separated chromatographically proved to be an important step in the solution of this problem. Using a column of silicone-treated kieselguhr and a solvent system of n-butanol, water, and citrate buffer at pH 4, Billlng (46) showed that the diazotization product of crystalline bilirubin (azopigment A) is relatively insoluble in water and, hence, moves slowly; in contrast tbat of Pigment II (azopigment B) is water-soluble and moves rapidly, while that of Pigment I behaves as a mixture of azopigments A and B. Subsequently, she pointed out that, because of the asymmetrical arrangement of the methyl and vinyl side chains in the bile pigments, each of the azopigments is made up of a mixture of two isomers differing only in the position of these side chains, and that although the isomers can be separated chromatographically their behavior is so similiar that they may be considered together as if they were one substance (47).

In 1956, by a remarkable coincidence, three groups of investigators working independently in England (48), in the United States (49), and in Czechoslovakia (50) simultaneously established that the direct-reacting bile pigment is a glucuronide of bilirubin. Billing, Cole & Lathe (48, 51) isolated a relatively pure preparation of azopigment B from diazotized human bile by the countercurrent distribution tecbnique, and showed that on treatment with alkali or on enzymatic hydrolysis with β-glucuronidase it was converted to azopigment A with the release of a reducing substance which was identified chromatographically as glucuronic acid. By direct measurement, it was found that the latter was present in the original azopigment B in a molar ratio of 1:1, indicating a monoglucuronide structure. Since bilirubin yields

two molecules of azopigment on diazotization, these investigators concluded that Pigment II, which gives rise to azopigment B exclusively, is a diglucuronide of bilirubin, and that Pigment I, which yields both azopigment A and B, is a monoglucuronide. They suggested, moreover, that an ester linkage between glucuronic acid and the proprionic acid side chains was more likely than a glycosidic linkage through the hydroxyl groups of bilirubin, in view of the ease with which the conjugates were converted to free bilirubin by mild alkali treatment. Subsequently, Schachter (52) confirmed this impression by demonstrating that the direct-reacting pigment in the urine of patients with obstructive jaundice and the azopigment B derived from it yield hydroxamic acids on treatment with hydroxyalamine, a reaction characteristic of carboxyl glucuronides. The structural relationships between bilirubin and its glucuronides and their respective azopigment derivatives are shown graphically in Figure 4.

Schmid (49, 53) isolated azopigments A and B from diazotized samples of icteric serum, urine, and bile by ascending paper chromatography, using a solvent system of ethyl methyl ketone, *n*-proprionic acid, and water. Since, on acid hydrolysis or incubation with β-glucuronidase, azopigment B yielded equimolar amounts of azopigment A and glucuronic acid, while azopigment A underwent no change, he reached the same conclusion regarding the structure of the direct-reacting bile pigments as had Billing and her associates (51). Talafant (50) also suggested that the latter are glucuronide conjugates of bilirubin, since he found that on paper electrophoresis of partially purified dog bile, the direct-reacting pigment fraction was constantly associated with glucuronic acid in a molar ratio of approximately 1:2.

Billing and her associates (51) found that from 10 to 15 per cent of the direct-reacting pigment in human bile was alkali-stable, suggesting that this fraction was not a glucuronide of bilirubin. Recently, Isselbacher & McCarthy (54) have confirmed this observation and shown chromatographically that approximately 24 per cent of the total azopigment derivatives of the bilirubin in human bile resist hydrolysis by β-glucuronidase, and that approximately 14 per cent is in the form of a sulfate. They suggest that the remaining 9 to 10 per cent of the alkali-labile pigment that contains neither glucuronide nor sulfate may be a carboxyl-linked methyl or glycine conjugate of bilirubin. Since the alkali-stable pigment isolated by these investigators possessed a free carboxyl group, as evidenced by its failure to react with hydroxalamine, and yielded a methyl derivative on treatment with diazo methane, they concluded that the sulfate conjugate is linked with one or both hydroxyl groups of bilirubin.

The rate at which bilirubin diazotizes appears to be dependent upon its solubility. Undoubtedly, this accounts for the prompt direct reaction given by its conjugates which are freely soluble in water at the pH of the van den Bergh reaction. In contrast, unconjugated bilirubin is only negligibly soluble in water at this pH (23), and thus reacts so slowly that color development is weak and greatly delayed visually, although it is readily detected spectro-

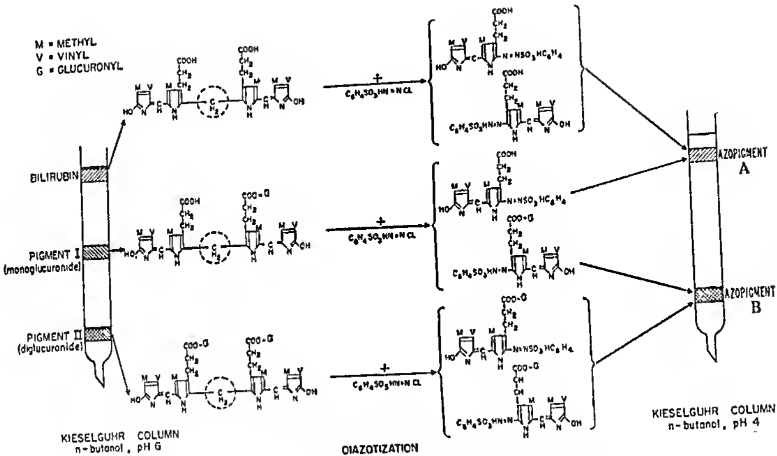


FIG. 4. Chromatographic behavior of and structural relationships between bilirubin, its glucuronides and their respective azopigment derivatives.

photometrically (55). Presumably, alcohol facilitates its prompt diazotization by increasing its solubility, since other methods that facilitate solubility of unconjugated bilirubin in the van den Bergh reaction mixture such as bile salts (56) or any solvent miscible in both water and chloroform (57), raising the pH of the reaction mixture (58) or esterifying the bilirubin with taurine (59), are as effective as alcohol. Billing and her associates (51) have found that ethanol is required for the complete coupling of Pigment I eluted from kieselguhr columns. However, Schachter (60) reports that, at least in plasma, bilirubin monoglucuronide couples completely in the absence of ethanol.

Since chromatographic methods for fractionating bilirubin in body fluids are too cumbersome for routine use clinically, colorimetic measurement of the direct van den Bergh reaction is still widely employed in estimating the concentration of conjugated pigment. Despite the fact that the latter method was devised long before the conjugates of bilirubin were isolated and characterized, and the fact that the results are influenced by the time allowed for coupling (61), the concentration of unconjugated bilirubin (55), the pH (62), the protein concentration (62), and the amount of diazotized sulfanilic acid used (62), it provides a reasonable approximation of the total amount of conjugated bilirubin estimated chromatographically. Some uncertainty still exists regarding the optimal time allowed for coupling. Billing (44) has found that the values obtained colorimetrically in the direct van den Bergh reaction at the end of 30 min., the interval recommended by Malloy & Evelyn (63), correlate more closely with the results of chromatography than those obtained at the end of 1 min., the interval recommended by Ducci & Watson (64). However, as pointed out by Watson (65), the preliminary separation of the bile pigments from protein required in the chromatographic method results in some loss of conjugated pigment in the protein precipitate, so that it cannot be used for evaluating the accuracy of other methods. Obviously the problem of the correct coupling time in the direct van den Bergh reaction will not be resolved until careful studies of the kinetics of the reaction have been carried out on purified samples of the bilirubin conjugates under conditions obtaining in the body fluids.

Normal serum contains a small fraction, usually 0.2 mg. per 100 ml. or less, that reacts directly within 1 min. in the van den Bergh reaction (64). It is not clear whether this represents conjugated pigment or is the fraction of unconjugated bilirubin that couples in the absence of alcohol. The fact that the injection of crystalline bilirubin into healthy subjects results in a delayed rise in the direct-reacting pigment of the serum that exceeds the small rise (approximately 3 per cent) when bilirubin is added to serum *in vitro*, suggests that under normal conditions small amounts of conjugated pigment may regurgitate into the blood from the bile. Unfortunately, it has not been possible to isolate and characterize this fraction chromatographically, since the method is insensitive to such low concentrations.

Enzymatic conjugation of bilirubin.—Soon after the structure of direct-reacting bilirubin was identified, a number of workers (37, 57, 66, 67) re-

ported that the conjugation of bilirubin with glucuronic acid involves an enzyme system in the liver that is similar to, or identical with, that responsible for the conjugation of a wide variety of other alcoholic and phenolic compounds (68). These investigators found that the incubation of bilirubin with liver slices immersed in a phosphate-bicarbonate medium containing serum (37), or with liver homogenates (37, 57, 67) or microsomes (37, 57, 66, 67) in a mixture of buffer, $MgCl_2$ and boiled liver extract, resulted in the formation of bilirubin glucuronide, which was identified by its direct van den Bergh reaction (37, 57, 66, 67), its hydrolysis by β-glucuronidase (57, 66), the chromatographic behavior of its azopigment derivatives (57, 66, 67), and the demonstration of free glucuronide following acid hydrolysis of the latter (57, 66, 67). It is reasonably certain from these observations, considered in the light of previous work reviewed by Isselbacher (68), that in the process of conjugation glucuronic acid, derived from uridine diphosphate glucuronic acid (UDPGA), a heat-stable nucleotide that can be extracted from boiled liver, is transferred to the carboxyl groups of bilirubin by means of glucuronyl transferase, an enzyme system found in the microsomes of the liver. As can be seen from the following scheme, glucose serves as the source of glucuronic acid in this system, several intermediate enzymatic reactions being involved:

$$\text{(i)}\quad \text{Glucose} + \text{ATP} \xrightleftharpoons{\text{hexokinase}} \text{Glucose-6-P}$$

$$\text{(ii)}\quad \text{Glucose-6-P} \xrightleftharpoons{\text{phosphoglucomutase}} \text{Glucose-1-P}$$

$$\text{(iii)}\quad \text{Glucose-1-P} + \text{UTP} \xrightleftharpoons[\text{pyrophosphorylase}]{\text{UDPG}} \text{UDPG} + \text{PP}$$

$$\text{(iv)}\quad \text{UDPG} + 2\text{DPN}^+ \xrightarrow[\text{dehydrogenase}]{\text{UDPG}} \text{UDPGA} + 2\text{DPNH} + 2\text{H}^+$$

$$\text{(v)}\quad \text{UDPGA} + \text{Bilirubin} \xrightarrow[\text{transferase}]{\text{glucuronyl}} \text{Bilirubin glucuronide} + \text{UDP}$$

That bilirubin may be conjugated directly with glucuronic acid by an alternative pathway is considered possible by some investigators. Danoff *et al.* (69) have reported that the administration of glucuronic acid to infants with increased serum levels of unconjugated bilirubin associated with erythroblastosis or physiological jaundice results in lowering of the level, an effect interpreted as possibly attributable to extrahepatic conjugation of the pigment with infused glucuronide. Johnson and her associates (70) have observed a similiar phenomenon in young rats with indirect hyperbilirubinemia caused by a hereditary deficiency of glucuronyl transferase (34), but note that the fall in serum bilirubin does not prevent kernicterus and usually is accompanied by increased icterus of the body fat, suggesting that the fall in serum bilirubin may result from, in part at least, a shift of pigment to the

tissues. A similar shift of pigment from the serum to the spinal fluid and muscles following intravenous sodium glucuronate has been observed in normal rabbits infused with bilirubin (71). Driscoll *et al.* (72) report that the administration to dogs of borneol with glucuronolactone-1-C^{14} results in labeling of a small fraction of the borneol glucuronide recovered from the urine, an effect that is not seen when glucuronolactone-6-C^{14} is used. They conclude that glucuronolactone-1-C^{14} is not incorporated directly, but undergoes degradation to fragments that condense to form glucose, which ultimately makes its way into UDPGA. Brown *et al.* (73) have found that liver homogenates from newborn guinea pigs deficient in glucuronyl transferase are capable of conjugating *o*-aminophenol in the presence of glucuronic acid, sodium glucuronate, or glucuronolactone, provided ATP is added. Since β-glucuronidase, an enzyme known to be present in high concentration in the newborn, decreases the conjugation of *o*-aminophenol when UDPGA serves as the glucuronide donor, and since this effect is reversed by both sodium glucuronate and known β-glucuronidase inhibitors, these authors suggest that glucuronic acid may enhance the activity of the UDPGA-glucuronyl transferase system by inhibiting β-glucuronidase. This appears unlikely in view of the observation that in the absence of glucuronyl transferase, 4-methyl umbelliferone will conjugate with glucuronic acid when incubated with ATP, UTP, and a soluble fraction of rat liver homogenate, an effect interpreted by Arias and his colleagues (74) as evidence supporting an alternative pathway of glucuronide conjugation. Whether or not this pathway is operative *in vivo* remains to be determined, but certainly the preponderance of evidence suggests that the major, and possibly the only, mechanism for glucuronide conjugation is the previously described shift of glucuronic acid from UDPGA to a suitable receptor by glucuronyl transferase.

The problem of whether or not the glucuronyl transferase involved in the conjugation of bilirubin is specific has not been resolved. Lathe & Walker (37) report that among different animals there is no parallelism between the *in vitro* capacities of the liver to conjugate bilirubin and *o*-aminophenol. Also, as shown by Arias & Johnson (32), the infusion of N-acetyl-*p*-aminophenol, another glucuronide receptor, does not competitively inhibit the conjugation of bilirubin *in vivo*, as judged by the plasma clearance and biliary excretion of injected bilirubin. These findings suggest that bilirubin and other aglycones may require different transferases for conjugation. However, a number of observations on the response of the liver to other glucuronide-binding compounds support the contrary view that the activity of the enzyme may be non-specific. Thus, (*a*) borneol inhibits the synthesis of bilirubin glucuronide by rat liver homogenates (57); (*b*) the liver of the Gunn rat with an hereditary deficiency of bilirubin glucuronyl transferase activity shows a parallel defect in its capacity to conjugate *o*-aminobenzoic acid and menthol *in vivo* and *o*-aminophenol *in vitro* (34, 75); and (*c*) children and adults with congenital non-hemolytic jaundice of the Crigler-Najjar type

(76) and the same hereditary enzymatic deficiency seen in the Gunn rat (77), show impairment of glucuronide conjugation with N-acetyl-*p*-aminophenol (77), tetrahydrocortisone (77, 78), trichlorethanol (78), and sodium salicylate (78). Lathe & Walker (37) suggest that the inhibition of bilirubin conjugation by borneol observed by Grodsky & Carbone (57) may have been caused by competition for a limited amount of UDPGA rather than for glucuronyl transferase. Also, the possibility must be considered that in the Gunn rat and in individuals with the Crigler-Najjar type of congenital jaundice, the hereditary defect may involve more than one enzyme. Clearly, the question regarding the specificity of bilirubin glucuronide transferase cannot be answered at this time, and must await the outcome of studies with more purified preparations of the enzyme system than are currently available.

Isselbacher & McCarthy (54) have succeeded in conjugating bilirubin with sulfate *in vitro* in a medium containing an ammonium sulfate fraction of liver and ATP. This suggests that the sulfate of bilirubin found in bile is synthesized enzymatically, and that the "active sulfate," adenosine-3′ phosphate-5′ phosphosulfate, may be an intermediate in the reaction. On treatment of bilirubin with acetic anhydride and sulfuric acid in the absence of any enzyme, a water-soluble, direct-reacting sulfate is formed (79). However, Isselbacher & McCarthy (54) have shown that this reaction involves a change in the structure of the bilirubin moiety, so that the synthetic pigment formed bears no relationship to the bilirubin sulfate conjugate normally found in bile.

Extrahepatic conjugation of bilirubin.—Following total hepatectomy in the dog, increasing amounts of unconjugated bilirubin and Pigment I can be detected in the serum (80). Since simultaneous nephrectomy does not prevent the accumulation of Pigment I (80), it must be assumed that the conjugation of bilirubin occurs in some extrahepatic site other than the kidney. Similar results have been obtained in the rat not only after hepatectomy and nephrectomy, but also after complete evisceration (80). However, in the rat a small amount of Pigment II accumulates when the kidneys are left *in situ*, but not when they are removed, suggesting that in this species some conjugation may occur in the kidney, at least after hepatectomy (80). The injection of bilirubin or hemoglobin into hepatectomized dogs or rats does not alter the ratio of Pigment I to total bilirubin in the serum, but the same type of injection into intact animals leads to an increase in the biliary excretion of Pigment I (80), an observation that has been interpreted as evidence that Pigment I, in part at least, is formed in the liver (80). On the basis of these observations, Hoffman and his associates (80) have advanced the hypothesis (*a*) that the conjugation of bilirubin to Pigment I occurs both in the liver and in extrahepatic tissues and (*b*) that Pigment I is converted to Pigment II within the liver, although in the rat the kidney may share this function to a minor degree, at least under the artificial conditions of total hepatectomy. Billing (45), who has studied the serum pigments in patients with jaundice caused by biliary obstruction and hepatitis, is in agreement with the view

that Pigment I is an intermediate in the formation of Pigment II from bilirubin, and that this step in the conjugation occurs in the liver. Unfortunately, in none of the experiments on hepatectomized animals cited was it established biochemically that Pigment I and the traces of Pigment II found in the serum were the monoglucuronide and diglucuronide of bilirubin, respectively. Since glucuronide and non-glucuronide conjugates cannot be differentiated by the chromatographic methods used in these experiments (48, 54, 59), the possibility cannot be excluded that the conjugates of bilirubin formed following hepatectomy were not glucuronides.

Grodsky & Carbone (57) have shown that *in vitro* kidney and, to a lesser extent, brain but not other tissues, are capable of conjugating bilirubin to a direct-reacting pigment in the presence of boiled liver extract. However, in these experiments, too, it was not established that the conjugate formed was a glucuronide. Since kidney tissue can conjugate other aglycones with glucuronide (81), it is reasonable to assume that it can do so with bilirubin, but there is a real need to establish this point unequivocally, since it may be a factor to be considered in interpreting the significance of the monoglucuronide of bilirubin found in the serum of jaundiced patients.

Using 4-methyl umbelliferone as a glucuronide receptor, Arias, Lowy & London (74) have demonstrated the presence of glucuronyl transferase in the serum under conditions of hepatic necrosis, both in animals and in man. That this enzyme is capable of conjugating bilirubin has been confirmed in *in vitro* experiments on serum obtained from rats given carbon tetrachloride (82). If it can be established that the enzyme is also active *in vivo*, either in the circulation or in tissues other than the liver, this may account, in part, for the paradoxically high levels of conjugated bilirubin found in the serum in hepatic necrosis when glucuronyl transferase activity in the liver is depressed (83). Of interest in this connection is the observation that the decline in hepatic transferase activity that follows liver damage results from a loss of enzyme-containing microsomes rather than from a reduction in their enzyme content (83). This raises the question of whether the transferase activity demonstrable in the serum is attributable to circulating microsomes or to the presence of the enzyme in some solubilized form.

Fate of bilirubin.—In the intestinal tract, bilirubin, as a consequence of bacterial action, undergoes a series of reductive reactions leading to the formation of two groups of compounds known collectively as the "urobilinogens" (84). These include (*a*) the colorless urobilinogens which characteristically react with Ehrlich's aldehyde reagent to yield red aldehyde complexes, and (*b*) their colored oxidation products, the urobilins, which, on mixing with Schlesinger's solution (zinc acetate in alcohol), yield zinc complexes with an intense green fluorescence. As illustrated in Figure 5, bilirubin is reduced successively to mesobilirubin, dihydromesobilirubin, and finally to the two colorless urobilinogens, mesobilirubinogen and stercobilinogen. To a variable degree, these are then oxidized to their respective urobilins, i-urobilin that is optically inactive, and l-stercobilin that is levorotatory. The precise

onstration that when N^{15} stercobilin is fed to animals made anemic by bleeding none of the label appears in newly synthesized hemoglobin (96). Studies conducted *in vitro* (97) and *in vivo* (91) suggest that some of the reabsorbed urobilinogen may be destroyed in the liver, but from the experiments of Mann & Koler (91) it would appear that this would not account for more than a small fraction of the pigment. With (98) has observed that, under conditions of sustained jaundice, non-bilirubin pigments appear in the serum, and has proposed that these represent dipyrryl end products of bilirubin degradation in the tissues. Since bilirubin is so readily cleared from the blood by the liver, it is not likely that it is destroyed in the tissues normally. However, this may be one of the mechanisms that tend to stabilize the serum, bilirubin level under conditions of prolonged jaundice. The most attractive hypothesis that would best explain the previously mentioned discrepancies is that there is an alternative pathway for the degradation of hemoglobin that does not involve the formation of bilirubin. No direct evidence can be cited in support of this possibility, but the observations of Katz, Ducci & Alessandri (99) on the effects of adrenocortical steroids in obstructive jaundice may be pertinent. These investigators found that when cortisone or prednisone was administered to patients with complete biliary obstruction there was a significant decrease in the concentration of bilirubin in the serum and in the amount excreted in the urine, but the output of urobilinogen in the feces remained constant at a low level. Although the possibility that the steroids retarded the rate of red cell destruction, or inhibited the breakdown of hemoglobin in the reticuloendothelium was not excluded in these experiments, the findings are consistent with the view that under some conditions hemoglobin may be degraded to colorless compounds that do not couple with diazotized sulfanilic acid. Further studies along these lines are needed to establish this point.

BILE PIGMENT METABOLISM IN JAUNDICE

When bilirubin enters the circulation more rapidly than it is removed, it accumulates in the plasma and tissues, ultimately staining the latter to produce jaundice. Although this may occur under a wide variety of circumstances, only three basic mechanisms, either singly or in combination, are ever involved: (*a*) increased production of bilirubin, (*b*) impairment of the liver's capacity to take up, conjugate, or excrete bilirubin, and (*c*) regurgitation of bilirubin into the plasma from the bile. A classification of jaundice based on the nature of the underlying disturbance in pigment metabolism would appear to be the most logical. However, it has the disadvantage that the basic mechanism involved is not always known, and that the multiplicity of factors in many cases would make it cumbersome and confusing. Accordingly, for clinical purposes it is more convenient and informative to classify jaundice on the basis of its pathogenesis, that is, as being of hemolytic, hepatocellular, or biliary obstructive origin, although, as will be evident from

the discussion to follow, some forms of jaundice do not lend themselves to this classification and must be considered separately.

Surprisingly little is known about the process of tissue staining in jaundice. However, there is some evidence to indicate that it entails a transfer of bilirubin from plasma albumin to tissue proteins, and especially to those of elastic tissue (100). As shown by Klein (101), this can be demonstrated *in vivo* by comparing the features of equal-sized wheals induced in the skin of patients with mild hyperbilirubinemia by injecting histamine in one site and the patient's own serum in another. Invariably the histamine-induced wheal becomes more deeply icteric, develops a higher bilirubin content, and remains pigmented longer. It is unlikely that the difference is attributable to a higher concentration of serum albumin in the histamine wheal, since application of a tourniquet above the site of histamine injection prevents local bile staining but not wheal formation. By the same technique, it has been shown that the skin stains more readily with direct-reacting bilirubin than the indirect-reacting type (101), which is in accord with the clinical observation that, at any given serum level of bilirubin, jaundice tends to be more intense in obstructive than in hemolytic jaundice; presumably this arises from the greater solubility of bilirubin glucuronide in the body fluids. The brain, in contrast to other tissues, at least in the newborn with kernicterus, appears to have a greater avidity for unconjugated bilirubin, a circumstance generally attributed to the greater solubility of the latter in lipids (102). Recently, it has been shown that, in the course of exchange transfusions in infants with marked non-hemolytic hyperbilirubinemia, more bilirubin is removed than can be accounted for in the circulation (103, 104). It is evident, therefore, that bilirubin in the tissues promptly returns to the circulation as its concentration in the plasma falls.

Bilirubinuria.—The factors governing the renal excretion of bilirubin are poorly understood. Normally, the urine contains no bilirubin although under some circumstances traces are detectable at normal serum levels (61). Characteristically, bilirubin appears in the urine when its concentration in the plasma is raised by biliary obstruction or hepatocellular disease, but not when the hyperbilirubinemia is of hemolytic origin. This difference has been attributed to the fact that the serum bilirubin is conjugated in the former and unconjugated in the latter, on the assumption that because the glucuronides are more soluble in water they are more readily excreted (49, 51). Consistent with this interpretation is the observation that the bilirubin found in urine is predominantly of the conjugated type (49, 51). However, the fact that the amount excreted is not closely correlated with the concentration of direct-reacting pigment in the serum (61) suggests that other factors may be involved. In this connection it is noteworthy that at the onset of viral hepatitis bilirubin may appear in the urine at minimally elevated levels of direct-reacting bilirubin in the serum, while during convalescence it often fails to do so at levels several times higher (105). Possibly, discrepancies such as these

can be accounted for on the basis of Schachter's observation that the renal clearance of the diglucuronide of bilirubin is greater than that of its monoglucuronide (60). Bilirubinuria is seen occasionally in hemolytic jaundice, and occurs with regularity in normal subjects infused with unconjugated crystalline bilirubin (55). This does not necessarily imply that the latter is excreted directly since, under both circumstances, a small fraction of direct-reacting pigment can be detected in the serum (55).

The renal clearance of bilirubin is very low. In Schachter's study (60) it ranged between 0.05 and 0.16 ml. per minute for the monoglucuronide, and from 0.41 to 0.96 ml. per minute for the diglucuronide. Attempts to investigate the renal excretory mechanism involved have led to conflicting results, some indicating that bilirubin is excreted by glomerular filtration (106), others that it is excreted by the tubules (107). All of these studies were carried out before it was appreciated that the clearance rates for bilirubin and its conjugates differ, so that these studies must be repeated before any valid conclusions can be reached. In the homozygous Gunn rat with hereditary jaundice, crystals of unconjugated bilirubin tend to accumulate in the tip of the renal papilla (34); this may be attributable to precipitation of pigment as a consequence of oversaturation of the local tissue proteins or to other local environmental changes favoring the separation of the pigment from protein. Taken together with the recent report that the bilirubin found in urine is bound to a mucoprotein (108), the finding of bilirubin crystals in the papilla suggests the possibility that the more soluble glucuronides of bilirubin may be excreted by the tubules in this region.

Bilirubin in cerebrospinal fluid.—Under appropriate conditions bilirubin readily gains access to the plasma, lymph, and urine, but rarely is found in any of the remaining body fluids or secretions other than the cerebrospinal fluid (107).

In the adult small amounts of direct- and indirect-reacting bilirubin may appear in the cerebrospinal fluid (CSF) in either hepatocellular or obstructive jaundice particularly when the serum bilirubin is high (109). This is an inconstant finding and is not dependent upon any increase in CSF protein (109).

Most newborn infants exhibit an increase in the serum concentration of unconjugated bilirubin shortly after birth (*vide infra*). According to Roberts (110), a similar but considerably smaller increase occurs in the CSF with the same degree of regularity. Stempfel & Zetterström (111) have confirmed this observation in infants with "physiological" and hemolytic jaundice, and have shown that the increase in CSF bilirubin parallels a rise in the CSF protein level. They have attributed these findings to functional immaturity of the newborn infant's blood:brain barrier resulting in a relative increase in its permeability to protein and unconjugated bilirubin. This appears reasonable in view of the demonstration that the intraperitoneal injection of bilirubin produces convulsions and bile-staining of the brain in newborn rats, but not in adult animals (112), and that increasing the permeability of the barrier by

intracisternal injections of *p*-chloromercuribenzoate in rabbits facilitates the transfer of protein and unconjugated bilirubin into the CSF (113).

Kernicterus.—Newborn infants with high levels of unconjugated bilirubin in the serum are subject to a neurological disorder that usually makes its appearance on the second day of life and often is fatal (114). Characteristically, pigmentation and degenerative changes are demonstrable in the basal ganglia and other areas of the brain. The pigment in these areas has been identified as unconjugated bilirubin (115, 116). Since it has been shown that the occurrence of kernicterus is closely correlated with the level of unconjugated bilirubin in the serum (114), that lowering the level by exchange transfusion usually prevents its development (114), and that the pigment is capable of uncoupling oxidative phosphorylation in the isolated mitochondria of liver and brain tissue (113) and of depressing the oxygen uptake of minced brain (117), it is generally agreed that the neurological lesions are probably caused by the direct toxic effect of bilirubin. The readiness with which unconjugated bilirubin gains access to brain tissue has been attributed to its lipid solubility (102) and affinity for certain brain lipids (115), and to the relative permeability of the blood:brain barrier to this pigment in the newborn (113, 118). Although traces of conjugated bilirubin have been identified in the brain lesions (115), kernicterus has not been encountered in newborn infants with very high levels in the serum (115). The *in vitro* effects of conjugated bilirubin on brain tissue have not been investigated. Accordingly, the question of whether or not this pigment can penetrate and injure the brain has not been resolved. However, this is of academic interest only since high serum levels of conjugated bilirubin are rarely encountered during the first few days of life when the infant is susceptible to kernicterus.

Kernicterus occurs not only in association with the increased hemolysis of isoimmunization, but also under other conditions that (*a*) favor the accumulation of unconjugated bilirubin, such as immaturity of the bilirubin-conjugating system of the liver (*vide infra*), liver damage, and other types of hemolysis; (*b*) increase the permeability of the blood:brain barrier; or (*c*) favor the dissociation of bilirubin from albumin in the plasma. The premature infant is particularly susceptible (119, 120) probably because of the immaturity of both its bilirubin-conjugating system (121, 122) and blood:brain barrier (113, 118). Of interest in this connection is the fact that the Gunn rat with a hereditary deficiency of glucuronyl transferase also develops typical kernicterus (70). The administration of large doses of some water-soluble vitamin K analogues to premature infants (123) raises the serum bilirubin level in these infants and increases their susceptibility to kernicterus. Although the precise mechanism has not been established, it is known that these agents induce hemolysis (124) and hepatic injury (125) in animals. Premature infants given sulfisoxazole also show an enhanced susceptibility to kernicterus, but the latter usually develops at relatively low levels of serum bilirubin (126). In the light of Odell's work (28), it is highly probable that this agent interferes with the binding of bilirubin by serum albumin and thus

phalein through the hepatic cells and then alters their permeability, resulting in the regurgitation of bile from the canaliculi. This is the first successful attempt to reproduce cholestatic jaundice in animals. With this model it should be possible to study the mechanism involved in even greater detail.

The centrilobular coarsely granular pigment in the Dubin-Johnson syndrome resembles the lipochromes but tends to be coarser and shows slightly different staining properties (137). In the writer's experience, these granules, except for their size, are indistinguishable from those seen in normal livers as described by Post and his associates (147). Of particular interest in this connection is the report of Wolf *et al.* (148) that in two families with the Dubin-Johnson syndrome the amount of pigment found in affected members varied considerably and in some was indistinguishable from that found in normal livers. This suggests that the Dubin-Johnson pigment may represent normal pigment that has accumulated as a result of the same defect in transport responsible for the retention of bilirubin. Also, it brings up the interesting possibility that the Rotor syndrome may be a variant of the Dubin-Johnson syndrome in which pigment fails to accumulate.

Although the occurrence of the Dubin-Johnson and Rotor syndromes in families is well documented, the hereditary nature of these disorders has by no means been established. The possibility that at least some are acquired and that environmental factors may be involved, has not been excluded. In this connection it is noteworthy that the Dubin-Johnson syndrome occurs sporadically more often than it does as a familial disorder (137), that not infrequently the onset is acute with symptoms suggestive of viral hepatitis even in familial cases (149), and that the disease may have its onset very late in life (150).

Jaundice with Predominantly Unconjugated Bilirubin in the Serum

Hemolytic jaundice.—If the rate of bilirubin production is accelerated by increased hemolysis, the pigment load may ultimately exceed the excretory capacity of the liver, resulting in bilirubin retention and jaundice. According to some authorities (128), the normal functional reserve of the liver is so great that rarely, if ever, is bilirubin retained as a result of hemolysis unless liver function is impaired. Estimates of the relative capacities for bilirubin production and excretion support this view in part, but indicate that, under some circumstances, it is possible to raise the serum bilirubin level slightly by increasing the pigment load in the absence of hepatic dysfunction. Weech and his associates (29) found that the velocity constant for bilirubin excretion in most normal subjects ranges from 5 to 10×10^{-3} mg. per minute per mg.[2] of plasma concentration per 100 ml. of plasma. From this it may be estimated that a normal adult with a plasma volume of 3 l. and a bilirubin concentration of 1.0 mg. per 100 ml. is capable of excreting 220 to 440 mg. of bilirubin per day. Considering that the normal production of bilirubin is ap-

jaundice. These appear to be inappropriate, since the available evidence favors the view that the defect is in the hepatic cells rather than in the cholangioles or canaliculi, and that it involves a functional disturbance in the transport system and possibly the permeability of the cells. That such disturbances can occur in cells showing no histological abnormalities has been clearly demonstrated by Hanzon (35).

Partition chromatography of the serum bile pigments in chlorpromazine jaundice (143), the Dubin-Johnson syndrome (144), and the Rotor syndrome (138) has revealed increased concentrations of all three types of bilirubin with a preponderance of Pigment I. As previously noted (*vide supra*), this pigment pattern is consistent with a disturbance in hepatocellular function. Since the patients with the Dubin-Johnson and Rotor syndromes show a normal increase in glucuronide excretion following test doses of salicylates (138, 144), the disturbance does not appear to involve the glucuronyl tranferase system of their hepatic cells. Taken together with the fact that their bilirubin clearance is impaired (138) and that their levels of serum bile acids and alkaline phosphatase are within normal limits (138, 144), these observations support the view that the defect in the Dubin-Johnson and Rotor syndromes is in the transport mechanism of the hepatic cells. The situation may be somewhat different in cholestatic jaundice arising from drug reactions and viral hepatitis since, under these conditions, the serum levels of alkaline phosphatase and bile acids tend to rise. This suggests that, in addition to a defect in the transport mechanism, there may be an increase in hepatic cell permeability permitting reflux of bile from the canaliculi. According to Hanzon (35), the initial effect of injury in the hepatic cell is the impairment of active transport which, if the injury is sufficiently severe, is followed by an alteration in cell permeability. It is quite possible, therefore, that further study of patients with cholestatic jaundice will reveal varying degrees of these two defects depending upon the severity of hepatocellular damage. These defects probably account also for the impaired excretion of bromsulphalein and other dyes so commonly observed in this group of disorders.

It is of interest that a similar type of cholestatic jaundice, known as "Geeldikkop," occurs in South African sheep that feed on a poisonous plant, *Lippia rehmanni* (145). Recently, Rimington and his associates (146) have studied the effects of icterogenin, a triterpene acid derived from the plant, on bile pigment metabolism. They found that on injecting the material intraperitoneally into rabbits with cannulated bile ducts there was (*a*) a marked decrease in bile volume; (*b*) suppression of bilirubin and bromsulphalein excretion; (*c*) an increase in Pigment I in the serum; and (*d*) an inability on the part of the liver to excrete intravenously administered conjugates of bilirubin and bromsulphalein. In contrast, the biliary excretion of alkaline phosphatase and bile acids was not affected and the liver showed no histological abnormalities. On the basis of these observations, Rimington concluded that icterogenin retards the transport of bilirubin and bromsul-

phalein through the hepatic cells and then alters their permeability, resulting in the regurgitation of bile from the canaliculi. This is the first successful attempt to reproduce cholestatic jaundice in animals. With this model it should be possible to study the mechanism involved in even greater detail.

The centrilobular coarsely granular pigment in the Dubin-Johnson syndrome resembles the lipochromes but tends to be coarser and shows slightly different staining properties (137). In the writer's experience, these granules, except for their size, are indistinguishable from those seen in normal livers as described by Post and his associates (147). Of particular interest in this connection is the report of Wolf *et al.* (148) that in two families with the Dubin-Johnson syndrome the amount of pigment found in affected members varied considerably and in some was indistinguishable from that found in normal livers. This suggests that the Dubin-Johnson pigment may represent normal pigment that has accumulated as a result of the same defect in transport responsible for the retention of bilirubin. Also, it brings up the interesting possibility that the Rotor syndrome may be a variant of the Dubin-Johnson syndrome in which pigment fails to accumulate.

Although the occurrence of the Dubin-Johnson and Rotor syndromes in families is well documented, the hereditary nature of these disorders has by no means been established. The possibility that at least some are acquired and that environmental factors may be involved, has not been excluded. In this connection it is noteworthy that the Dubin-Johnson syndrome occurs sporadically more often than it does as a familial disorder (137), that not infrequently the onset is acute with symptoms suggestive of viral hepatitis even in familial cases (149), and that the disease may have its onset very late in life (150).

Jaundice with Predominantly Unconjugated Bilirubin in the Serum

Hemolytic jaundice.—If the rate of bilirubin production is accelerated by increased hemolysis, the pigment load may ultimately exceed the excretory capacity of the liver, resulting in bilirubin retention and jaundice. According to some authorities (128), the normal functional reserve of the liver is so great that rarely, if ever, is bilirubin retained as a result of hemolysis unless liver function is impaired. Estimates of the relative capacities for bilirubin production and excretion support this view in part, but indicate that, under some circumstances, it is possible to raise the serum bilirubin level slightly by increasing the pigment load in the absence of hepatic dysfunction. Weech and his associates (29) found that the velocity constant for bilirubin excretion in most normal subjects ranges from 5 to 10×10^{-3} mg. per minute per mg.[3] of plasma concentration per 100 ml. of plasma. From this it may be estimated that a normal adult with a plasma volume of 3 l. and a bilirubin concentration of 1.0 mg. per 100 ml. is capable of excreting 220 to 440 mg. of bilirubin per day. Considering that the normal production of bilirubin is ap-

lirubinemia reached during the neonatal period, especially in the premature infant. These have been alluded to in the section on kernicterus, and are considered in detail in a recent review by Lucey (120).

Non-hemolytic "overproduction" jaundice.—Hyperbilirubinemia of the indirect-reacting type and mild jaundice are common findings in pernicious anemia (7). Since the life span of the red cells is shorter than normal (164) and the excretion of urobilinogen is abnormally high (7), it is generally agreed that increased hemolysis is one of the factors involved in the retention of bilirubin in this disease. However, as pointed out previously, at least 40 per cent of the bile pigment excreted by individuals so affected is derived from sources other than the hemoglobin of circulating erythrocytes (20). It is quite possible, therefore, that overproduction of bilirubin unrelated to hemolysis may contribute to the development of jaundice in pernicious anemia.

Recently, Israels and his associates (165) have invoked the same mechanism to account for an unusual form of indirect-reacting hyperbilirubinemia which they encountered in four individuals, three of whom were members of the same family. They termed the disorder "shunt hyperbilirubinemia," emphasizing the concept that the overproduction of bilirubin in these cases occurred over some pathway that did not involve the breakdown of hemoglobin in circulating erythrocytes. The features of the disorder which, in all four cases, had its onset during the second decade, were indistinguishable from those of congenital hemolytic jaundice and included indirect-reacting hyperbilirubinemia, spherocytosis, reticulocytosis, increased osmotic fragility, normoblastic hyperplasia of the marrow, a shortened Cr^{51} red cell survival time, greatly increased fecal urobilinogen excretion, and splenomegaly. However, the response to splenectomy was quite atypical in that it abolished excessive hemolysis, as evidenced by a return of the Cr^{51} red cell survival time to normal, but failed to correct any of the other abnormalities. In contrast to the usual postsplenectomy findings in congenital hemolytic jaundice, these patients continued to exhibit hyperbilirubinemia and a high output of urobilinogen in the apparent absence of excessive red cell destruction, suggesting an overproduction of bilirubin from some other source. In one case, it was estimated that the latter accounted for no less than 75 per cent of the total pigment excreted.

Although the evidence presented makes it highly improbable that the excess bilirubin found in Israels' patients was derived from circulating erythrocytes, it is perhaps a bit premature to speak of a "shunt" mechanism, which implies an alternative pathway for bilirubin production, since the bilirubin may have been derived from the hemoglobin of erythrocytes or younger red cells destroyed in the bone marrow. In view of the typical hematological and biochemical features they exhibited, it is difficult to exclude the possibility that these patients had classical congenital spherocytic hemolytic jaundice. If such was the case, the persistence of normoblastosis in the marrow and

lirubinemia reached during the neonatal period, especially in the premature infant. These have been alluded to in the section on kernicterus, and are considered in detail in a recent review by Lucey (120).

Non-hemolytic "overproduction" jaundice.—Hyperbilirubinemia of the indirect-reacting type and mild jaundice are common findings in pernicious anemia (7). Since the life span of the red cells is shorter than normal (164) and the excretion of urobilinogen is abnormally high (7), it is generally agreed that increased hemolysis is one of the factors involved in the retention of bilirubin in this disease. However, as pointed out previously, at least 40 per cent of the bile pigment excreted by individuals so affected is derived from sources other than the hemoglobin of circulating erythrocytes (20). It is quite possible, therefore, that overproduction of bilirubin unrelated to hemolysis may contribute to the development of jaundice in pernicious anemia.

Recently, Israels and his associates (165) have invoked the same mechanism to account for an unusual form of indirect-reacting hyperbilirubinemia which they encountered in four individuals, three of whom were members of the same family. They termed the disorder "shunt hyperbilirubinemia," emphasizing the concept that the overproduction of bilirubin in these cases occurred over some pathway that did not involve the breakdown of hemoglobin in circulating erythrocytes. The features of the disorder which, in all four cases, had its onset during the second decade, were indistinguishable from those of congenital hemolytic jaundice and included indirect-reacting hyperbilirubinemia, spherocytosis, reticulocytosis, increased osmotic fragility, normoblastic hyperplasia of the marrow, a shortened Cr^{51} red cell survival time, greatly increased fecal urobilinogen excretion, and splenomegaly. However, the response to splenectomy was quite atypical in that it abolished excessive hemolysis, as evidenced by a return of the Cr^{51} red cell survival time to normal, but failed to correct any of the other abnormalities. In contrast to the usual postsplenectomy findings in congenital hemolytic jaundice, these patients continued to exhibit hyperbilirubinemia and a high output of urobilinogen in the apparent absence of excessive red cell destruction, suggesting an overproduction of bilirubin from some otber source. In one case, it was estimated that the latter accouoted for no less than 75 per cent of the total pigment excreted.

Although the evidence presented makes it highly improbable that the excess bilirubin found in Israels' patients was derived from circulating erythrocytes, it is perhaps a bit premature to speak of a "shuot" mechanism, which implies an alternative pathway for bilirubin production, since the bilirubio may have been derived from the hemoglobin of erythrocytes or younger red cells destroyed in the bone marrow. In view of the typical hematological and biochemical features they exhibited, it is difficult to exclude the possibility that these patients had classical congenital spherocytic hemolytic jaundice. If such was the case, the persistence of normoblastosis in the marrow and

reticulocytosis in the peripheral blood after splenectomy would have to be interpreted as evidence of continued excessive hemolysis. The only way this could occur without lowering the Cr^{51} red cell survival time would be if erythrocytes or younger red cells were destroyed in the marrow before getting into the circulation. Of course, this same line of reasoning could be applied in the case of pernicious anemia and congenital porphyria, so that it is by no means established that the bilirubin derived from sources other than the circulating erythrocytes is produced by a process that does not involve the degradation of hemoglobin.

Production of bilirubin in excess of that expected from the destruction of circulating erythrocytes may be a more common phenomenon in hemolytic and other disorders associated with erythroid hyperplasia than is generally recognized. Conceivably, this is one of the mechanisms responsible for the type of non-hemolytic hyperbilirubinemia seen occasionally in association with hepatosplenomegaly and increased urobilinogen excretion following viral hepatitis (165, 166). However, since the clearance rates for bilirubin and bromsulphalein usually are reduced in such cases, it is evident that impairment of excretion also plays a role.

Familial and hereditary forms of non-hemolytic, non-obstructive jaundice.—Three familial disorders characterized by retention in the plasma of unconjugated bilirubin in the absence of increased hemolysis or overt liver disease have been described: (*a*) congenital familial non-hemolytic jaundice with kernicterus [Crigler & Najjar (76)]; (*b*) transient familial hyperbilirubinemia [Lucey & Driscoll (167)]; and (*c*) constitutional hepatic dysfunction, also known as Gilbert's syndrome, simple familial cholemia, icterus intermittens juvenilis, hereditary non-hemolytic bilirubinemia, and familial non-hemolytic jaundice (168). To avoid this confusing and cumbersome terminology, these are designated as the Crigler-Najjar, Lucey-Driscoll, and Gilbert syndromes, respectively, for the purposes of this discussion.

The hereditary nature of the Crigler-Najjar syndrome has been established (76, 78). Although the Lucey-Driscoll syndrome is seen in successive newborn infants of certain women, there is as yet no evidence to indicate that its occurrence is necessarily genetically determined (169). It is clear from recent studies (168, 170) that a number of disorders, only some of which are familial, may give rise to Gilbert's syndrome. For that reason, Gilbert's syndrome will be considered separately.

tensely jaundiced with a serum bilirubin level of approximately 25 mg. per 100 ml., he was otherwise well and had developed normally. In the same report, Childs & Najjar describe still another case from a fourth related family, a two-year-old child who had been icteric since birth, but who was otherwise well. Rosenthal and his associates (172) have studied a six-year-old boy with the same disorder in whom signs of kernicterus did not appear until the age of three. In the case reported by Jervis (173), intense jaundice and neurological signs consistent with kernicterus became apparent in infancy, and then persisted until the patient's death at the age of 44. There was no family history of jaundice in this instance, but the paternity of the patient was in doubt. For reasons that will be evident later, it is quite possible that the two cases reported by Arias & London (67), as instances of Gilbert's syndrome with high levels of bilirubin in the serum, also belong in this category.

Investigation of patients with the Crigler-Najjar syndrome has demonstrated that their jaundice results from impairment of bilirubin excretion as a consequence of an hereditary glucuronyl transferase deficiency. Thus, it has been shown that homogenates of liver from affected individuals fail to conjugate bilirubin *in vitro* (173), and that the ability of such patients to form glucuronides *in vivo* with other aglycones such as salicylates (78), N-acetyl-*p*-aminophenol (77), trichlorethanol (78), and tetrahydrocortisone (77, 78), is impaired. Other observations that support this interpretation are: (*a*) the only pigment found in the serum is unconjugated bilirubin (77); (*b*) no conjugated bilirubin is detectable in the bile (77); (*c*) bilirubin tolerance is greatly impaired (76); (*d*) there is no hematological or biochemical evidence of increased hemolysis (76); and (*e*) both functionally and histologically the liver is normal (76).

Although the parents of children with the Crigler-Najjar syndrome exhibit neither hyperbilirubinemia nor neurological abnormalities, their ability to conjugate test substances, such as salicylates, with glucuronic acid usually is impaired, but not as greatly as in their affected children (78). This has led Childs and his associates (78) to propose that (*a*) the Crigler-Najjar syndrome is genetically determined; (*b*) individuals affected with the disease are homozygous while both their parents are heterozygous for the mutant gene; and (*c*) the gene is recessive with respect to jaundice but shows incomplete dominance with respect to glucuronide conjugation of test substances.

The Lucey-Driscoll syndrome (167) is a form of intense but transient jaundice that affects newborn infants of mothers who, although apparently normal in other respects, have generally produced more than one infant with the same disorder. Characteristically, the serum bilirubin is of the indirect-reacting type and reaches very high levels, usually between 20 and 60 mg. per 100 ml. Kernicterus is a common complication unless exchange transfusions are carried out. In infants who survive, the serum bilirubin falls to a normal level within the first month of life. In many respects, this jaundice resembles neonatal jaundice but none of the factors known to predispose to

such high levels of serum bilirubin and to the development of kernicterus, such as prematurity, vitamin K therapy, sepsis, or isoimmunization with hemolysis, have been implicated. Arias & Wolfson (169) have found that the serum in affected children and in their mothers during pregnancy inhibit bilirubin conjugation by rat liver slices three to five times as effectively as the serum of normal pregnant women and their newborn infants. Conceivably, such inhibition is of significance in the etiology of the disease. In view of Lathe & Walker's work (161), the possibility must be considered that the inhibitor is a steroid that appears in the blood during normal pregnancy but accumulates in higher concentration in those women who give birth to infants with the Lucey-Driscoll syndrome.

Gilbert's syndrome.—The distinguishing feature of this disorder is a mild, chronic, apparently non-hemolytic hyperbilirubinemia of the indirect-reacting type (168, 174). Occasionally, it is familial in character but more often it is not. Usually the jaundice is first recognized in adolescence or in early adult life, but the onset may occur in infancy or late life. The serum level of bilirubin tends to be low, usually less than 5 mg. per 100 ml., but very high levels have been reported (67). Fluctuations in the level are common and may, in some instances, be attributable to intercurrent infections, excessive alcohol intake, or fatigue. According to some authorities (174), the serum bilirubin concentration tends to fall with advancing age but others (168) have observed sustained values for many years. Often the jaundice is discovered during some unrelated illness or in the course of a routine physical examination. Fatigability, asthenia, and gastrointestinal complaints are said to be common and often wax and wane with the serum bilirubin level but, in the writer's experience, the disorder tends to be asymptomatic unless the patient has some unrelated associated disease or has become anxious over the possibility that the jaundice is a manifestation of some serious chronic disorder of the liver. Except for impaired bilirubin tolerance, hepatic function is normal. Histologically, the liver shows no abnormalities although mild fatty infiltration is seen in some cases (175). Since, by definition, the hyperbilirubinemia is non-hemolytic, most authors exclude cases with reticulocytosis, increased fecal urobilinogen, or splenomegaly. Yet, the Cr^{51} red cell survival time has been found slightly reduced, suggesting increased hemolysis, in some individuals classified as having Gilbert's syndrome (168).

On chromatographic analysis, the serum pigment has been identified as unconjugated bilirubin (168), while the pigment found in the bile has been shown to be a mixture of Pigments I and II with the latter predominating, as in normal bile (67, 168). Studies of the glucuronide conjugating mechanism in the liver have led to contradictory results. In two patients with serum bilirubin levels of 8.8 and 18.8 mg. per 100 ml., respectively, Arias & London (67) demonstrated impairment of *in vitro* conjugation of bilirubin by homogenates and microsome preparations of liver biopsy specimens in the presence of UDPGA. Accordingly, they concluded that the hyperbilirubi-

nemia of Gilbert's syndrome is the consequence of impaired bilirubin excretion attributable to a deficiency of glucuronyl transferase in the liver. Subsequently Arias, Lowy & London (74) confirmed these observatioos in six similar patients with serum bilirubin levels ranging between 1.8 and 22 mg. per 100 ml.; io this study they used the conjugation of 4-methyl umbelliferone with glucuronide by homogenates of needle biopsy specimens of the liver as an index of glucuronyl transferase activity. In contrast, neither Schmid & Hammaker (176), usiog N-acetyl-*p*-aminophenol, nor Foulk and his colleagues (168), using menthol, were able to demonstrate any defect in the *in vivo* conjugation of these compounds with glucuronide in patients with relatively low levels of serum bilirubin. Schmid & Hammaker (176) concluded that the defect in Gilbert's syndrome is not a deficiency of glucuronyl transferase, but may involve a faulty mechanism for the transport of bilirubin from the plasma to the site of the conjugating enzyme within the hepatic cells. They pointed out that the serum bilirubin levels in Arias and London's cases were very much higher than those usually seen in Gilbert's syndrome, and suggested that these may have been examples of the Crigler-Najjar syndrome. Although Schiff & Billiog (177) agree with the view that the glucuronyl transferase system is normal in Gilbert's syndrome particularly in view of the predominance of Pigment II in the bile and the normal excretion of urobilinogen in the feces, they point out that *in vivo* tests with ethereal-forming glucuronide receptors, such as N-acetyl-*p*-aminophenol and menthol, may not be a valid measure of bilirubin glucuronyl transferase activity, since the latter entails the formation of an ester glucuronide. In a recent report, Arias (170) has attempted to reconcile these conflictiog viewpoints. He suggests that Gilbert's syndrome comprises a heterogeneous group of disorders, only one of which is associated with a deficiency of glucuronyl transferase. The latter is probably a non-lethal variant of the Crigler-Najjar syndrome without kernicterus, has its onset in childhood, and usually is accompanied by serum levels of bilirubin in excess of 5 mg. per 100 ml., although occasionally lower levels are seen. In the remaining cases of varied etiology, glucuronyl transferase activity is normal.

What conclusions are to be drawn about the etiology and pathogenesis of Gilbert's syndrome depends entirely upon how the disorder is defined. If the term is used broadly to include all instances of indirect-hyperbilirubinemia not attributable to overt hemolysis, which appears reasonable in view of the ill-defined nature of the original cases described by Gilbert, it may be properly applied to (*a*) the Crigler-Najjar syndrome in infants and its less severe counterpart in adults, as described by Arias & London (67); (*b*) the hyperbilirubinemia seen following viral hepatitis (166, 178) and in association with other diseases of the hepatobiliary system (168) caused probably by a defect in the transport mechanism of the hepatic parenchymal cells as suggested by Schmid (176); (*c*) compensated hemolytic disease; in this group the reduction in the red cell life span usually encountered (168) is far too little to

account for hyperbilirubinemia so that it is highly probable that there is an additional defect in bilirubin transport; and (*d*) overproduction of bilirubin in the bone marrow in association with the hyperplasia of the marrow seen in some cases of compensated hemolytic disease, as described by Israels *et al.* (165). As previously mentioned, the possibility that the latter mechanism is involved also in the hyperbilirubinemia seen in association with splenomegaly and increased fecal urobilinogen excretion following viral hepatitis (166), cannot be excluded.

It would be highly desirable to abandon the term "Gilbert's syndrome," and use a nomenclature based on the nature of the underlying defects involved. Although it is not always possible to do so except in the case of a deficiency of glucuronyl transferase, an attempt should be made in all cases to exclude genetic factors, an enzymatic defect, underlying hepatic or biliary disease, or an undetected compensated hemolytic disorder.

LITERATURE CITED

1. London, I. M., Shemin, D., and Rittenberg, D., *J. Clin. Invest.*, 27, 547 (1948)
2. Rich, A. R., *Physiol. Revs.*, 5, 182 (1925)
3. Gubler, C. G., *Science*, 123, 87 (1956)
4. Miller, L. L., Robscheit-Robbins, F. S., and Whipple, G. H., *J. Exptl. Med.*, 81, 405 (1945)
5. Fiessinger, N., Gaydos, A., and Polonovski, M., *Compt. rend. soc. biol.*, 135, 1572 (1941)
6. Klatskin, G., and Bungards, L., *J. Clin. Invest.*, 35, 537 (1956)
7. Watson, C. J., *Downey's Handbook of Hematology*, IV, 2447 (Paul B. Hoeber, Inc., New York, N. Y., 1938)
8. Kench, J. E., *Biochem. J.*, 56, 669 (1954)
9. London, I. M., *J. Biol. Chem.*, 184, 373 (1950)
10. Fairley, H. H., *Brit. Med. J.*, II, 95 (1940)
11. Bingold, K., *Folia Haematol.*, 42, 192 (1930)
12. London, I. M., West, R., Shemin, D., and Rittenberg, D., *J. Biol. Chem.*, 184, 351 (1950)
13. Gray, C. H., Neuberger, A., and Sneath, P. H. A., *Biochem. J.*, 47, 87 (1950)
14. Watson, C. J., Pass, I. J., and Schwartz, S., *J. Biol. Chem.*, 139, 583 (1941)
15. London, I. M., Yamasaki, M., and Sabella, G., *Federation Proc.*, 10, 217 (1951)
16. Lemberg, R., *Revs. Pure and Appl. Chem.*, 6, 1 (1956)
17. Lemberg, R., and Legge, J. W., *Hematin Compounds and Bile Pigments* (Interscience Publishers, Inc., New York, N. Y., 1949)
18. Gardikas, C., Kench, J. E., and Wilkinson, J. F., *Biochem. J.*, 46, 85 (1950)
19. Crosby, W. H., *J. Clin. Invest.*, 37, 887 (1958)
20. London, I. M., and West, R., *J. Biol. Chem.*, 184, 359 (1950)
21. London, I. M., West, R., Shemin, D., and Rittenberg, D., *J. Biol. Chem.*, 184, 365 (1950)
22. Gray, C. H., *The Bile Pigments*, 101 (John Wiley & Sons, Inc., New York, N. Y., 1953)
23. Overbeek, J. T. G., Vink, C. L. J., and Deenstra, H., *Rec. trav. chim.*, 74, 81 (1955)
24. Martin, N. H., *J. Am. Chem. Soc.*, 71, 1230 (1949)
25. Gray, C. H., and Kekwick, R. A., *Nature*, 161, 274 (1948)
26. Cohn, E. J., *Blood*, 3, 471 (1948)
27. Snapper, I., and Bendien, W. M., *Acta Med. Scand.*, 98, 77 (1938)
28. Odell, G. B., *J. Clin. Invest.*, 38, 823 (1959)
29. Weech, A. A., *J. Clin. Invest.*, 20, 323 (1941)
30. Zieve, L., Hill, E., Hanson, M., Falcone, A. B., and Watson, C. J., *J. Lab. Clin. Med.*, 38, 446 (1951)
31. Dragstedt, C. A., and Millo, M. A., *Am. J. Physiol.*, 119, 713 (1937)
32. Arias, I. M., and Johnson, L., *Clin. Research*, 7, 291 (1959)
33. Gunn, C. H., *J. Heredity*, 29, 137 (1938)
34. Schmid, R., Axelrod, J., Hammaker, L., and Swarm, R. L., *J. Clin. Invest.*, 37, 1123 (1958)
35. Hanzon, V., *Acta Physiol. Scand.*, 28, Suppl. 101 (1952)
36. Weinbren, K., and Billing, B. H., *Brit. J. Exptl. Pathol.*, 37, 199 (1956)
37. Lathe, G. H., and Walker, M., *Biochem. J.*, 70, 705 (1958)
38. Overbeek, J. T. G., Vink, C. L. J., and Deenstra, H., *Rec. trav. chim.*, 74, 85 (1955)
39. Ehrlich, P., *Centr. klin. Med.*, 4, 721 (1883)
40. van den Bergh, A. A. H., and Snapper, J., *Deut. Arch. klin. Med.*, 110, 540 (1913)
41. van den Bergh, A. A. H., and Müller, P., *Biochem. Z.*, 77, 90 (1916)
42. Cole, P. G., and Lathe, G. H., *J. Clin. Pathol.*, 6, 99 (1953)
43. Cole, P. G., Lathe, G. H., and Billing, B. H., *Biochem. J.*, 57, 514 (1954)
44. Billing, B. H., *J. Clin. Pathol.*, 8, 126 (1955)
45. Billing, B. H., *J. Clin. Pathol.*, 8, 130 (1955)
46. Billing, B. H., *Biochem. J.*, 56 (1954)
47. Billing, B. H., and Lathe, G. H., *Am. J. Med.*, 24, 111 (1958)
48. Billing, B. H., and Lathe, G. H., *Biochem. J.*, 63, 6p (1956)
49. Schmid, R., *Science*, 124, 76 (1956)
50. Talafant, E., *Collection trav. chim. tchécoslov.*, 22, 661 (1957)
51. Billing, B. H., Cole, P. G., and Lathe, G. H., *Biochem. J.*, 65, 774 (1957)
52. Schachter, D., *Science*, 126, 507 (1957)
53. Schmid, R., *J. Biol. Chem.*, 229, 881 (1957)

54. Isselbacher, K. J., and McCarthy, E. A., *J. Clin. Invest.*, 38, 645 (1959)
55. Tisdale, W. A., Klatskin, G. and Kinsella, E. D., *Am. J. Med.*, 26, 214 (1959)
56. Kühn, H. A., *Z. ges. exptl. Med.*, 115, 371 (1950)
57. Grodsky, G. M., and Carbone, J. V., *J. Biol. Chem.*, 226, 449 (1957)
58. Gedigk, P., and Gries, G., *Z. physiol. Chem.*, 289, 261 (1952)
59. Jirsa, M., Večerek, B., Ledvina, M., *Nature*, 177, 895 (1956)
60. Schachter, D., *J. Lab. Clin. Med.*, 53, 557 (1959)
61. Klatskin, G., and Drill, V. A., *J. Clin. Invest.*, 29, 660 (1950)
62. Lathe, G. H., and Ruthven, C. R. J., *J. Clin. Pathol.*, 11, 155 (1958)
63. Malloy, H. T., and Evelyn, K. A., *J. Biol. Chem.*, 119, 481 (1937)
64. Ducci, H., and Watson, C. J., *J. Lab. Clin. Med.*, 30, 293 (1945)
65. Watson, C. J., *Ann. Internal Med.*, 45, 351 (1956)
66. Schmid, R., Hammaker, L., and Axelrod, J., *Arch. Biochem. Biophys.*, 70, 285 (1957)
67. Arias, I. M., and London, I. M., *Science*, 126, 563 (1957)
68. Isselbacher, K. J., *Recent Progr. in Hormone Research*, 12, 134 (1956)
69. Danoff, S., Grantz, C., Boyer, A., and Holt, L. E., Jr., *Science*, 127, 759 (1958)
70. Johnson, L., Sarmiento, F., Blanc, W. A., and Day, R., *J. Diseases Children*, 97, 591 (1959)
71. Lee, T. C., and Hsia, D. Y. Y., *J. Lab. Clin. Med.*, 54, 512 (1959)
72. Driscoll, S. G., Hsia, D. Y. Y., Dennen, D. A., and Dowben, R. M., *Am. J. Physiol.*, 197, 1322 (1959)
73. Brown, A. K., Zuelzer, W. W., and Bollett, A. J., *J. Diseases Children*, 96, 487 (1958)
74. Arias, I. M., Lowy, B. A., and London, I. M., *J. Clin. Invest.*, 37, 875 (1958)
75. Carbone, J. V., and Grodsky, G. M., *Proc. Soc. Exptl. Biol. Med.*, 94, 461 (1957)
76. Crigler, J. F., Jr., and Najjar, V. A., *Pediatrics*, 10, 169 (1952)
77. Axelrod, S., Schmid, R., and Hammaker, L., *Nature*, 180, 1426 (1957)
78. Childs, B., Sidbury, J. B., and Migeon, C. J., *Pediatrics*, 23, 903 (1959)
79. Watson, C. J., *Science*, 128, 142 (1958)
80. Hoffman, H. N., Whitcomb, F. F., Jr., Butt, H. R., and Bollman, J. L., *J. Clin. Invest.*, 39, 132 (1960)
81. Karunairatnam, M. C., Kerr, L. M. H., and Levvy, G. A., *Biochem. J.*, 45, 496 (1949)
82. Fenster, F., and Klatskin, G. (Unpublished data)
83. Isselbacher, K. J., and McCarthy, E. A., *Proc. Soc. Exptl. Biol. Med.*, 103, 819 (1960)
84. Watson, C. J., and Weimer, M., *J. Lab. Clin. Med.*, 54, 1 (1959)
85. Watson, C. J., Campbell, M., and Lowry, P. T., *Proc. Soc. Exptl. Biol. Med.*, 98, 707 (1958)
86. Watson, C. J., *Ann. Internal Med.*, 47, 611 (1957)
87. Sborov, V. M., Jay, A. R., and Watson, C. J., *J. Lab. Clin. Med.*, 37, 52 (1951)
88. Baumgärtl, T., *Fortsch. Med.*, 69, 181 (1951)
89. Hollan, O. R., *Gastroenterology*, 16, 418 (1950)
90. McMaster, R. D., and Elman, R. *J. Exptl. Med.*, 41, 719 (1925)
91. Mann, J. D., and Koler, R. D., *Gastroenterology*, 17, 400 (1951)
92. Watson, C. J., *Arch. Internal. Med.*, 59, 196 (1937)
93. Klatskin, G. (Unpublished data)
94. Bingold, K., and Stieb, W., *Ergeb. inn. Med. u. Kinderheilk.*, 5, 707 (1954)
95. Gilbertsen, A. S., Lowry, P. T., Hawkinson, V., and Watson, C. J., *J. Clin. Invest.*, 38, 1166 (1959)
96. Siegel, X. X., and Lowry, P. T., cited by Watson, C. J., *Ann. Internal Med.*, 47, (1957)
97. Felix, K., and Moebus, H., *Z. physiol. Chem.*, 236, 230 (1935)
98. With, T. K., *Acta Med. Scand.*, 122, 501 (1945)
99. Katz, R., Ducci, H., and Alessandri, H., *J. Clin. Invest.*, 36, 1370 (1957)
100. Rosenthal, F., *Klin. Wochschr.*, 9, 1909 (1930)
101. Klein, O., *Klin. Wochschr.*, 10, 2032 (1931)
102. Lathe, G. H., *Lancet*, II, 683 (1956)
103. Brown, A. K., Zuelzer, W. W., and Robinson, A. R., *J. Diseases Children* 93, 274 (1957)
104. Forfar, J. O., Keay, A. J., Elliott, W. D., and Cumming, R. E., *Lancet*, II, 1131 (1958)
105. Watson, C. J., and Hoffbauer, F. W., *Ann. Internal Med.*, 26, 813 (1947)
106. Halász, H., *Schweiz. med. Wochschr.*, 75, 220 (1945)
107. With, T. K., *Acta Physiol. Scand.*, 10, 355 (1945)
108. Heikel, T., Sipilä, A. M., and Teuhunen, R., *Scand. J. Lab. & Invest.*, 9, 342 (1957)

109. Rosenberg, D. G., and Galamboa, J. T., *Am. J. Digest. Diseases*, 5, 32 (1960)
110. Roberts, M. H., *Southern Med. J.*, 21, 460 (1928)
111. Stempfel, R., and Zetterström, R., *Pediatrics*, 16, 184 (1955)
112. Waters, W. J., and Britton, H. A., *Pediatrics*, 15, 45 (1955)
113. Ernster, L., Herlin, L., and Zetterström, R., *Pediatrics*, 20, 647 (1957)
114. Hsia, D. Y., Allen, F. H., Jr., Gellis, S. S., and Diamond, L. K., *New Engl. J. Med.*, 247, 668 (1952)
115. Claireaux, A. E., Cole, P. G., and Lathe, G. H., *Lancet*, II, 1226 (1953)
116. Waters, W. J., Richert, D. A., and Rawson, H. H., *Pediatrics*, 13, 319 (1954)
117. Day, R., *Pediatrics*, 17, 925 (1956)
118. Nasralla, M., Gawronska, E., and Hsia, D. Y., *J. Clin. Invest.*, 37, 1403 (1958)
119. Brown, A. K., and Zuelzer, W. W., *J. Diseases Children*, 93, 263 (1957)
120. Lucey, J. F., *Pediatrics*, 25, 690 (1960)
121. Brown, A. K., and Zuelzer, W. W., *J. Clin. Invest.*, 37, 332 (1958)
122. Grodsky, G. M., Carbone, J. V., and Fanska, R., *Proc. Soc. Exptl. Biol. Med.*, 97, 291 (1958)
123. Bound, J. P., and Telfer, T. P., *Lancet*, I, 720 (1956)
124. Moore, T., and Sharman, I. M., *Lancet*, I, 819 (1955)
125. Richards, R. K., and Shapiro, S., *J. Pharmacol. Exptl. Therap.*, 84, 93 (1945)
126. Harris, R. C., Lucey, J. F., and MacLean, R. J., *Pediatrics*, 21, 875 (1958)
127. Day, R., and Johnson, L., *Progr. in Hematol.*, 11, 133 (1959)
128. Rich, A. R., *Bull. Johns Hopkins Hosp.*, 47, 338 (1930)
129. Hiyeda, K., *Beitr. pathol. Anat. u. allgem. Pathol.*, 73, 541 (1925)
130. Baikie, A. G., *Scot. Med. J.*, 2, 359 (1957)
131. Isselbacher, K. J., *Gastroenterology*, 36, 1327 (1959)
132. Werther, J. L., and Korelitz, B. I., *Am. J. Med.*, 22, 351 (1957)
133. Werner, S. C., Hanger, F. M., and Kritzler, R. A., *Am. J. Med.*, 8, 325 (1950)
134. Schaffner, F., Popper, H., and Chesrow, E., *Am. J. Med.*, 26, 249 (1959)
135. Watson, C. J., and Hoffbauer, F. W., *Ann. Internal. Med.*, 25, 195 (1946)
136. Svanborg, A., and Ohlsson, S., *Am. J. Med.*, 27, 40 (1959)
137. Dubin, I. N., and Johnson, F. B., *Medicine*, 33, 155 (1954)
138. Schiff, L., Billings, B. H., and Oikawa, Y., *New Engl. J. Med.*, 260, 1315 (1959)
139. Tygstrup, N., *Lancet*, I, 1171 (1960)
140. Summerskill, W. H. J., and Walshe, J. M., *Lancet*, II, 686 (1959)
141. Steigmann, F., and Popper, H., *Gastroenterology*, 1, 645 (1943)
142. Dickes, R., Schenker, V., and Deutsch, L., *New Engl. J. Med.*, 256, 1 (1957)
143. Bollmann, J. L., *Gastroenterology*, 36, 1321 (1959)
144. Burka, E. R., *Ann. Internal Med.*, 52, 453 (1960)
145. Rimington, C., *Onderstepoort, J. Vet. Research*, 9, 225 (1937); *Lancet*, I, 772 (1955)
146. Rimington, C., Heikel, T., Knight, B. C., Wilhams, E. J., and Ritchie, H. D., *Gastroenterology*, 38, 796 (1960)
147. Post, J., Benton, J. G., and Breakstone, R., *Arch. Pathol.*, 52, 67 (1951)
148. Wolf, R. L., Pizette, M., Richman, A., Dreiling, D. A., Jacobs, W., Fernandez, O., and Popper, H., *Am. J. Med.*, 28, 32 (1960)
149. John, G. J., and Knudison, K. P., *Am. J. Med.*, 21, 138 (1956)
150. Bartholomew, L. G., Dearing, W. H., and Baggenstoss, A. H., *Gastroenterology*, 33, 302 (1957)
151. Passorelli, N. M., *Studies on the origin and Excretion of Conjugated Bilirubin in Bilirubin-Infused Rats* (Doctoral thesis, Yale University School of Medicine, New Haven, Conn., 1959)
152. Hsia, D. Y., Patterson, P., Allen, F. H., Jr., Diamond, L. K., and Gellis, S. S., *Pediatrics*, 10, 243 (1952)
153. Craig, J. M., *Arch. Pathol.*, 49, 665 (1950)
154. Weech, A. A., *Advances in Pediat.*, 10, 346 (1947)
155. Hsia, D. Y., Allen, F. H., Jr., Diamond, L. K., and Gellis, S. S., *J. Pediat.*, 42, 277 (1953)
156. Billing, B. H., Cole, P. G., and Lathe, G. H., *Brit. Med. J.*, II, 1263 (1954)
157. Anselmino, H. F., *Münch. med. Wochschr.*, 79, 1226 (1932)
158. Lathe, G. H., and Walker, M., *Biochem. J.*, 67, 9P (1957)
159. Vest, M. F., and Streiff, R. R., *J. Diseases Children*, 98, 688 (1959)
160. Schmid, R., Buckingham, S., Mendilla, G. A., and Hammaker, L., *Nature*, 183, 1823 (1959)

161. Lathe, G. H., and Walker, M., *Quart. J. Exptl. Physiol.*, 43, 257 (1958)
162. Fashena, G. J., Bates, H. H., and Reid, A. F., *J. Diseases Children*, 80, 510 (1950)
163. Mollison, P. L., *Lancet*, I, 513 (1948)
164. Hamilton, H. E., De Gowin, E. L., Sheets, R. F., Janney, C. D., and Ellis, J. E., *J. Clin. Invest.*, 33, 191 (1954)
165. Israels, L. G., Suderman, H. J., and Ritzman, S. E., *Am. J. Med.*, 27, 693 (1959)
166. Siede, W., *Deut. med. Wochschr.*, 82, 504 (1957)
167. Lucey, J. F., and Driscoll, T. J., *cited by* Arias, I. M., and Wolfson, S., *Gastroenterology*, 38, 797 (1960)
168. Foulk, W. T., Butt, H. R., Owen, C. A., Jr., Whitcomb, F. F., Jr., and Mason, H. L., *Medicine*, 38, 25 (1959)
169. Arias, I. M., and Wolfson, S., *Gastroenterology*, 38, 797 (1960)
170. Arias, I. M., *Med. Clin. N. Amer.*, 44, 607 (1960)
171. Childs, B., and Najjar, V. A., *Pediatrics*, 18, 369 (1956)
172. Rosenthal, I. M., and Zimmerman, H. J., *Trans. Am. Assoc. Study Liver Diseases* (Chicago, Ill., Nov. 3, 1955)
173. Jervis, G. A., *Arch. Neurol. Psychiat.*, 81, 55 (1959)
174. Meulengracht, E., *Quart. J. Med.*, 16, 83 (1947)
175. Hult, H., *Acta Med. Scand.*, 138, Suppl. 244 (1950)
176. Schmid, R., and Hammaker, L., *New Engl. J. Med.*, 260, 1310 (1959)
177. Schiff, L., and Billing, B. H., *Gastroenterology*, 37, 595 (1959)
178. Kalk, H., and Wildhirt, E., *Med. Klin. (Munich)*, 55, 694 (1960)

THE PORPHYRINS AND THE PORPHYRIAS[1,2]

By Lennox Eales, M.D., F.R.C.P.

Department of Medicine (C.S.I.R./U.C.T. Clinical Nutrition Unit), University of Cape Town, and Groote Schuur Hospital, Cape Town, South Africa

In the last decade there has been a great upsurge in interest not only in the biochemistry of the porphyrins but in the disorders of porphyrin metabolism. Since 1955, several good reviews (1 to 14) have appeared covering, with varying degrees of detail, the chemical and the clinical aspects of the porphyrins and the porphyrias. It is the purpose of this review to consider briefly our knowledge of porphyrin biosynthesis and its applications, and to discuss the classification and delineation of the various disorders of porphyrin metabolism. Particular attention will be given to the occurrence and manifestations of the porphyrias as seen in the Union of South Africa which can rate among its claims to fame the highest incidence of porphyria in the world. Before any discussion of the clinical aspects is possible, it is necessary to review our knowledge of the biosynthesis of the porphyrins.

Biosynthesis of the Porphyrins

Workers on both sides of the Atlantic have made major contributions. The Ciba Conference (14) provided a good insight into the development of our knowledge up to 1955, and highlighted the many points of difficulty. In addition to Shemin's (6) Harvey lecture and the detailed accounts of porphyrin and heme biosynthesis by Rimington (7, 8, 10), the enzyme systems involved in the porphyrin synthesis have been reviewed (11, 15).

Following the pioneer isotope studies of Shemin & Rittenberg (16, 17) who demonstrated that glycine was a direct precursor of the heme of haemoglobin, the origin of all the carbon and nitrogen atoms of the protoporphyrin molecule was established (4, 6). As a result of these elegant studies and those of Muir & Neuberger (18), glycine was shown to contribute all the nitrogen atoms, and the methene bridge carbon atoms, acetate through a 4 carbon compound, providing the remaining C atoms. The other precursor, "active" succinate, is derived from the citric acid cycle (4). In addition, Shemin *et al.* (19) postulated a succinate-glycine cycle, whereby glycine was utilised for the synthesis not only of δ-amino-laevulinic acid (ALA) but also of the purines. The steps in the biosynthetic sequence (see Fig. 1) may now be considered:

The formation of ALA by condensation of succinate and glycine.—The combination of 8 mol. of succinate with 8 mol. of glycine is necessary for the

[1] The survey of the literature pertaining to this review was concluded in May, 1960.

[2] The following abbreviations will be used: ALA (delta-amino-laevulinic acid); PBG (porphobilinogen); SH (sulfhydryl).

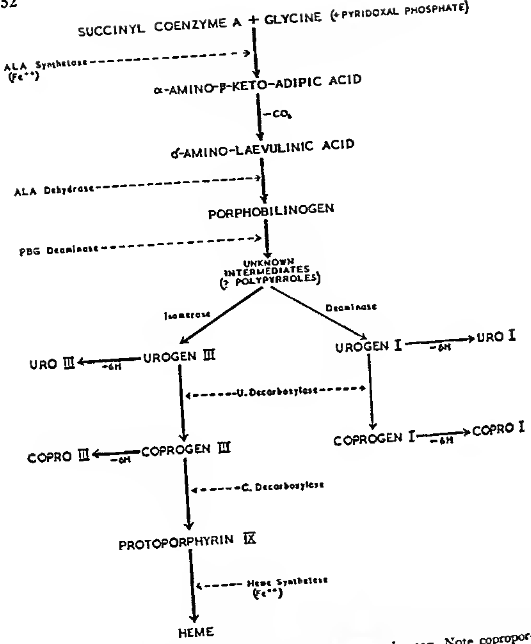


FIG. 1. Illustrating the possible biosynthetic sequence in man. Note coproporphyrinogen is decarboxylated and oxidised to protoporphyrinogen which undergoes auto-oxidation to protoporphyrin IX. (Uro-gen. = Uroporphyrinogen; Copro-gen = Coproporphyrinogen). (Uro = Uroporphyrin; Copro = Coproporphyrin).

synthesis of the porphyrin molecule. "Active" succinate condenses with glycine to give rise to α-amino-β-ketoadipic acid which decarboxylates (? enzymic) to ALA, and may then be utilised for porphyrin synthesis or alternatively metabolised (19) for synthesis of the ureido group of purines, etc., while the 4 carbon residue is reconverted to succinate. Haemolysates

from pyridoxine-deficient ducklings require pyridoxal phosphate for the synthesis of ALA from glycine and succinate (20), while intact chicken red cells require (21) a portion of the citric acid cycle and its coenzymes, an oxidative phosphorylating system, pyridoxal-phosphate, and glutamine. Other *in vitro* studies with particulate fractions of chicken red cells (22 to 24) and microbiological preparations (25, 26) have shown pyridoxal phosphate and coenzyme A to be necessary, while certain particulate-containing preparations synthesise ALA actively from glycine and succinyl coenzyme A (23) provided pyridoxal phosphate is present. It is probable that following activation of succinate by coenzyme A and ATP to form succinyl coenzyme A, condensation with a pyridoxal derivative of glycine occurs (27).
The enzyme(s) is bound to cell particulates and mitochondria are involved (28, 29).

The condensation of ALA to porphobilinogen.—The isolation of crystalline porphobilinogen (30) and the establishment of its chemical structure (31 to 33) was an important step. Radioactive labelling has shown that 2 mol. of ALA condense to form 1 mol. of PBG. That this conversion is enzymic was first demonstrated in a chicken red cell haemolysate system (34). The purified enzyme (ALA dehydrase) has been isolated from various animal, plant, and microbiological sources and has been extensively studied (35 to 39). Not only do whole blood and washed red cells show activity, but so also does a normal human leucocyte homogenate (40). It is an SH-containing enzyme (38), is inhibited by ethylenediamine tetracetic acid (38) and lead (39), and may contain copper (41).

The transformation of PBG to uroporphyrinogen.—There is good evidence that under ordinary conditions the enzymic conversion of 4 mol. PBG to 1 mol. of uroporphyrinogen III occurs. However, in some instances uroporphyrin III and "pseudo-uroporphyrin" (42) are formed. The claims that the latter is a true intermediate and not a chromatographic artefact (29) need confirmation. Preheating the enzyme system to 60° C. leads to formation of uroporphyrin I (43). The properties of the enzyme (porphobilinogenase) have been described (44).

Whereas, with various haemolysates, rapid conversion of PBG to uroporphyrin III occurs, with whole blood uroporphyrin I is formed, presumably because PBG cannot penetrate the cells (29). While Lockwood & Benson (45) could find no electrophoretic evidence of more than one enzyme, Granick & Mauzerall (38) have suggested a deaminase activity which condenses PBG to polypyrylmethanes by eliminating NH_3 and an isomerase activity which inverts one (or 3) PBG mol. to give a series III isomer, and it is this action which is destroyed by heat. Indeed, Bogorad (46) has succeeded in separating the two activities from plant extracts and has shown that deaminase action precedes isomerase action. Heath & Hoare's observations with a *Rhodopseudomonas spheroides* system (47) coincide with this finding.

It is difficult to explain the formation of the series III cyclic porphyrinogens from PBG. The mechanisms detailed by Rimington (10) [including the

proposed formation of di- and tri-pyrrylmethanes (51)], the possible participation of opsopyrrol-dicarboxylic acid (48) or the dipyrromethanes (49), and the recent ingenious postulation of an octapyrrol intermediate by Wittenberg (50) are all possible, but the nature of the intermediates is quite unknown. Mere demonstration of mechanisms *in vitro* carries no guarantee that these operate *in vivo*.

The transformation of uroporphyrinogen III to coproporphyrinogen III.—Uroporphyrinogen is the colourless reduced porphyrin containing 6 extra atoms of hydrogen. Uroporphyrin could not be used for heme or chlorophyll synthesis by either intact or disrupted cells (52 to 54), but uroporphyrinogen III is readily transformed to coproporphyrinogen III (55, 56). The transformations probably occur stepwise since, during enzymic decarboxylation (and oxidation) of uroporphyrinogen, porphyrins with 7, 6, and 5 carboxyl groups appear (57). Analogous fractions have been found frequently in urine of cases of porphyria. The enzyme(s) "uroporphyrinogen decarboxylase" decarboxylates both series I and III isomers. It contains SH groups. The formation of porphyrin depends on autoxidation and photocatalysis of the porphyrinogen, and *in vivo* this is normally unimportant.

Conversion of coproporphyrinogen III to protoporphyrin.—This necessitates the conversion of two of the propionic acid chains to 2 vinyl groups and is highly specific. There is no effect on coproporphyrin itself or on coproporphyrinogen I and this explains why only protoporphyrin 9, a series III isomer, is found in nature. The addition of rat liver mitochondria to human red cells incubated with PBG or ALA resulted in protoporphyrin formation at the expense of copro- and uroporphyrins (58), and evidence of direct conversion of coproporphyrinogen to protoporphyrin has been obtained (59). Since human and rabbit red cells lack activity as opposed to avian red cells and reticulocytes, this suggests that mitochondrial processes are active (28).

Incorporation of iron into protoprophyrin.—The conclusion that protoporphyrin is the direct precursor of heme is founded on sound experimental evidence. The enzymic conversion to heme is achieved by avian red cell haemolysates (60 to 63) and by rat liver (64). Chicken haemolysates and human reticulocytes showed rapid incorporation of C^{14}-tagged protoporphyrin 9 into heme (62). The enzyme (heme synthetase) contains SH groups and is inhibited by lead (65). Mitochondrial processes are involved (28, 66). A reducing agent is necessary for the activation of iron (67) and its incorporation into heme by a particulate supernatant of chicken haemolysate was potentiated by ascorbic acid, ergothioneine, and glutathione in concentrations that approximate those found normally in the erythrocytes (68). There is evidence that stroma iron of the red cell is an intermediate between transport iron of the plasma and the iron of haemoglobin (70). The release of iron from its linkage to transferrin, and its subsequent incorporation into hepatic ferritin is dependent on the oxidative metabolism of the liver cell (69).

While the notable contributions of the biochemists have greatly aided

our understanding of porphyrin biosynthesis, it must be remembered that the studies of the enzyme systems have been carried out *in vitro* under optimal and very particular conditions and, furthermore, with substrates divorced from the normal cell organisation with its mosaic of enzymes, and modifying factors. Apart from possible species differences, the diverse effects produced with different systems and substrates (29) is to be remembered. Thus, while an interpretation of the *in vitro* activity of a single enzyme is possible we must exercise caution in applying this knowledge to the intact animal. The sequence (Fig. 1) is well supported by experimental evidence and provides a working hypothesis. Moreover, with the development of suitable methods for the estimation of urinary ALA and PBG (71), all of the major intermediates of porphyrin biosynthesis—ALA, PBG, uro-, copro-, and protoporphyrin—have been identified in the excreta of normal man. Furthermore, in the various porphyrias the appearance of certain of these intermediates in greatly increased concentration in the excreta, and their presence in high concentration in certain tissues and organs, may be reasonably explained in terms of derangement of enzyme action at various points along the biosynthetic pathway.

THE PORPHYRIAS

The porphyrias may be defined as a group of disorders in which inborn or acquired derangements of enzyme action result in an increased excretion of the porphyrins or of their precursors, or both, in the excreta. The increased excetion is frequently, but not always, associated with a distinctive clinical syndrome. By convention, the term has been restricted to disease states in which considerable amounts of uroporphyrin or PBG appear in the urine. It has been customary to regard the porphyrias as inborn errors of metabolism and to deny the existence of acquired porphyria. That there may be an underlying susceptibility in the latter instance cannot be denied, but evidence of an inherited disorder is lacking. Particularly in cutaneous porphyria is this so. While the experimental induction of the disorder (72) and the frequency of exogeneous factors in human cases support the hypothesis of an acquired condition, Tio's (73, 74) remarkable case, and the recent "epidemic" of porphyria in Turkey (75) should dispel all doubts as to the possible existence of acquired porphyria. It has been customary to separate the coproporphyrinurias from the porphyrias but, if one is to be logical, the symptomatic porphyrinurias should be included in any consideration of the disorders of porphyrin metabolism and such cases may show evidence of disorder elsewhere in the biosynthetic chain. Thus, in lead intoxication apart from the gross coproporphyrinuria, an increased ALA excretion occurs (76, 76a, 77) and occasionally uroporphyrin (77) is found in the urine.

From our knowledge of the biosynthesis and the biochemical pattern of excretion in the various disorders, we can locate the probable sites of derangement but we are not yet in a position to speak of specific defects. Thus,

proposed formation of di- and tri-pyrrylmethanes (51)], the possible participation of opsopyrrol-dicarboxylic acid (48) or the dipyrromethanes (49), and the recent ingenious postulation of an octapyrrol intermediate by Wittenberg (50) are all possible, but the nature of the intermediates is quite unknown. Mere demonstration of mechanisms *in vitro* carries no guarantee that these operate *in vivo*.

The transformation of uroporphyrinogen III to coproporphyrinogen III.—Uroporphyrinogen is the colourless reduced porphyrin containing 6 extra atoms of hydrogen. Uroporphyrin could not be used for heme or chlorophyll synthesis by either intact or disrupted cells (52 to 54), but uroporphyrinogen III is readily transformed to coproporphyrinogen III (55, 56). The transformations probably occur stepwise since, during enzymic decarboxylation (and oxidation) of uroporphyrinogen, porphyrins with 7, 6, and 5 carboxyl groups appear (57). Analogous fractions have been found frequently in urine of cases of porphyria. The enzyme(s) "uroporphyrinogen decarboxylase" decarboxylates both series I and III isomers. It contains SH groups. The formation of porphyrin depends on autoxidation and photocatalysis of the porphyrinogen, and *in vivo* this is normally unimportant.

Conversion of coproporphyrinogen III to protoporphyrin.—This necessitates the conversion of two of the propionic acid chains to 2 vinyl groups and is highly specific. There is no effect on coproporphyrin itself or on coproporphyrinogen I and this explains why only protoporphyrin 9, a series III isomer, is found in nature. The addition of rat liver mitochondria to human red cells incubated with PBG or ALA resulted in protoporphyrin formation at the expense of copro- and uroporphyrins (58), and evidence of direct conversion of coproporphyrinogen to protoporphyrin has been obtained (59). Since human and rabbit red cells lack activity as opposed to avian red cells and reticulocytes, this suggests that mitochondrial processes are active (28).

Incorporation of iron into protoprophyrin.—The conclusion that protoporphyrin is the direct precursor of heme is founded on sound experimental evidence. The enzymic conversion to heme is achieved by avian red cell haemolysates (60 to 63) and by rat liver (64). Chicken haemolysates and human reticulocytes showed rapid incorporation of C^{14}-tagged protoporphyrin 9 into heme (62). The enzyme (heme synthetase) contains SH groups and is inhibited by lead (65). Mitochondrial processes are involved (28, 66). A reducing agent is necessary for the activation of iron (67) and its incorporation into heme by a particulate supernatant of chicken haemolysate was potentiated by ascorbic acid, ergothioneine, and glutathione in concentrations that approximate those found normally in the erythrocytes (68). There is evidence that stroma iron of the red cell is an intermediate between transport iron of the plasma and the iron of haemoglobin (70). The release of iron from its linkage to transferrin, and its subsequent incorporation into hepatic ferritin is dependent on the oxidative metabolism of the liver cell (69).

While the notable contributions of the biochemists have greatly aided

The Classification of the Porphyrias

Apart from the fact that there can be no entirely satisfactory classification until the precise biochemical disturbances in the various forms have been elucidated, the prime source of difficulty has arisen from the fact that in adults three recognisable clinical syndromes—the acute attack, the cutaneous syndrome, and a mixture of the two—have each in the past been equated with a disease when, in fact, the same syndrome may be the expression of two quite different disorders. This is illustrated by considering the incidence of the main clinical forms encountered in the racial groups in Cape Town (Fig. 2). While the three forms occur with equal frequency in the white race, the purely cutaneous form predominates in the Bantu and the Cape coloured races. The same cutaneous syndrome was seen in the three races but in the white race the majority of cases were attributed to protocoproporphyria, while in the Bantu all were cases of symptomatic cutaneous porphyria and in the Cape coloured race cutaneous cases resulting from both causes were observed.

Waldenström's (9) and Schmid *et al.*'s (103) classifications are com-

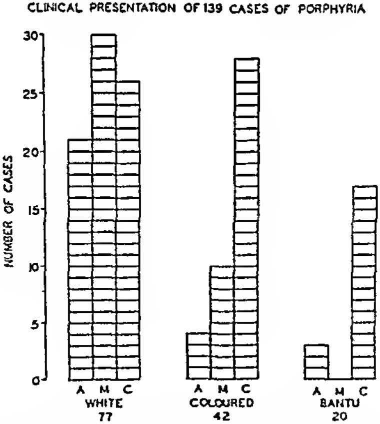


Fig. 2. Clinical presentation of porphyria in the 3 main racial groups in Cape Town. (From *S. African J. Lab. Clin. Med.*, 6, 63 1960.) A = Acute attack. C = Cutaneous syndrome. M = Acute attack *plus* cutaneous syndrome.

in erythropoietic porphyria, if we accept the hypothesis of a PBG deaminase and isomerase operating for the conversion of uroporphyrinogen III, then the condition may be explained by assuming that in the homozygous state one of the following abnormalities must exist: deficient isomerase activity, excessive PBG deaminase activity, or a combination of both. There is no evidence to support any of these possibilities. In acute intermittent porphyria the defect may be either a "block" in ALA and PBG conversion or an overproduction of ALA that exceeds the capacity of the porphobilinogenase system. A reduced liver catalase might be interpreted as indicating a possible site of block but, unlike experimental porphyria (75) in human porphyria (78), there is no fall in liver catalase and, furthermore, PBG formation appears to be increased beyond the normal requirements for heme synthesis. Overproduction of ALA may stem from increased synthesis from glycine and succinate, or from decreased metabolism along the purine pathway with diversion to the ALA side. In acute porphyria, ALA conversion to PBG is increased (79) and ALA dehydrase activity is presumably increased. In protocoproporphyria, in view of the constantly enhanced faecal excretion, the major defect appears to be at the protoporphyrin-coproporphyrin stages but the uroporphyrinogen decarboxylase system is usually also affected. Superimposed on this basic abnormality, there occurs a temporary disturbance of ALA and PBG formation, usually precipitated by the barbiturates and other drugs. In porphyria cutanea tarda symptomatica, presumably, uro- and coproporphyrinogen decarboxylases are affected, while in the coproporphyrinurias only the coproporphyrinogen decarboxylases are deranged.

Geographical Distribution and Incidence of the Porphyrias

Large series of cases of all types have been observed in several countries: United States of America, series of 255, 83, and 81 cases (80 to 82); Germany, 60 (12); Switzerland, 60 (83); England, 50 (84); France, 43 (85). Ippen (13) in his comprehensive review of cutaneous porphyria, notes cases from Northern, Southern, Eastern, Western, and Central Europe, North and South America, Asia, and Africa. While Waldenström's experience (9) of 321 cases of acute intermittent porphyria is unique, such cases have been reported from Ireland (86), Australia (87), New Zealand (88), and China (89). Pride of place, however, must go to the Union of South Africa where, among the white population, an inherited form of porphyria is extremely common. Following the first report in 1939 (90, 91), many cases have been seen. By 1951, Barnes (92) had already reported on 40 cases of "porphyrinuria," and a series of papers by Dean and Dean & Barnes (93 to 95) followed. That this may well become a national problem is indicated by Dean who claims to have diagnosed 564 cases of porphyria in 54 family groups (96). It is also seen in the Cape Coloured race (97, 98). "Many hundreds of cases" (99) of symptomatic cutaneous porphyria have been seen in the Bantu and have been reported from all parts of the country (98, 100 to 102).

1. Congenital Porphyria—Erythropoietic Porphyria
2. Acute Intermittent Porphyria—Pyrroloporphyria
3. Porphyria Cutanea Tarda Hereditaria—Protocoproporphyria
4. Porphyria Cutanea Tarda Symptomatica—Urocoproporphyria

The symptomatic porphyrinurias, both uroporphyrinuria and coproporphyrinuria, deserve inclusion. Uroporphyrinuria should probably be included under 4. It occurs in several conditions (110 to 112) and as a symptomless state in the Bantu (98) in wbom it may be associated with abnormal liver function tests (113). It is possibly the forerunner of cutaneous porphyria. Melanoderma-porphyria (114) may be an analogous condition. There are many causes of symptomatic coproporphyrinuria but the idiopathic and hereditary forms (115, 116) are known. Myoporphyria (83) is probably a variant of acute porphyria. Genuine mixed porphyria may occur if acute intermittent porphyria is combined with symptomatic porphyria, but it should be patent that in any case of acute intermittent porphyria or porphyria cutanea tarda symptomatica the differential diagnosis must include protocoproporphyria.

Erythropoietic porphyria:congenital porphyria.—The rarity of this disease is emphasised by the fact that Schmid *et al.* (116) were able to accept only 34 cases as genuine examples of this condition. Of Watson *et al.*'s (80) 255 cases, only 7 cases belong to this group, and 3 out of 43 (85) in another series. Stich added an additional 6 cases (12), and recently two cases in brothers from India (117) and a case in a Bantu girl (118) have been reported. Only one previous case in a Bantu is known (119) but there have been two cases in Sudanese siblings (120). The early onset of the cutaneous lesions, the erythrodontia without abdominal or neurological symptoms, and the red urine are sufficiently characteristic to make the diagnosis. The bone marrow contains numerous fluorocytes. The urine and the faeces contain porphyrin in large amounts. There is no porphobilinogenuria. The faecal porphyrin is largely coproporphyrin in contradistinction to the findings in protocoproporphyria (99). Since the first report from South Africa of a similar condition in cattle (121) many cases have been recorded and recently reviewed by Rimington (122). Watson *et al.* (80) have compared human and bovine erythropoietic porphyria. In bovines the condition is a recessive trait (122) as it almost certainly is in man. Both conditions are very similar but the erythrocyte porphyrins in the human were mainly uroporphyrin and in the bovine mainly protoporphyrin. An unexpected finding in the human case 246 was a very high hepatic porphyrin. The effect of splenectomy is unpredictable. Their case 71 has, however, remained latent for several years and urine porphyrin is one-third of presplenectomy levels.

Pyrroloporphyria:acute intermittent porphyria (Pyrrolia; Swedish genetic porphyria).—The incidence of this form has been estimated as 1/100,000 in Sweden (9) and 1.5/100,000 in Australia (123) while 8 overt and 4 latent cases have been observed in a population of 150,000 (124) in England. Most patients are of North European extraction but it has been reported in other

monly used but revision is needed. While there is general agreement on the clinical and pathological identity of erythropoietic porphyria, confusion has encompassed the porphyria hepatica subgroups, especially the "mixed" and the porphyria cutanea tarda varieties. Acute intermittent porphyria, too, has been confused repeatedly with the condition as seen in the white porphyric families in South Africa. Watson (104) has used the term "mixed porphyria" to cover those cases in which there were both cutaneous and acute symptoms, while cutanea tarda was reserved for the purely cutaneous cases. Unfortunately, many workers have used (and still use) the two terms synonymously, e.g., "porphyria cutaoea tarda or mixed porphyria as it is called by Watson" (105). Tio (73) rightly empbasised the importance of acquired porphyria and favoured Watson's classification but added to porphyria cutanea tarda the suffix "sensu strictiori" to denote the pure cutaneous syndrome. Clear evideoce of an inherited disorder in some cutaneous cases and not in others led Waldenström (9) to revise his original classification by subdividing the cutaneous cases into a hereditary and symptomatic group while he retained acute intermittent porphyria. Rimington (106) has supported Waldenström's classification strongly and decried Dean & Barnes' (96) suggestion of discarding acute intermittent porpbyria and replacing it with the Swedish aod South African types of porphyria hepatica. The unqualified title of cutanea tarda for the South African type is unacceptable since, in one and the same sibship, cases may manifest with acute attacks, with cutaneous symptoms, or with a combination of the two, or the sole evidence of porphyria may be an increased faecal porphyrin. This variety of presentation prompted Dean & Barnes (107) to suggest the term "porphyria variegata." Although tbis descriptive title has an unfortuoate horticultural flavour and was not received with any editorial enthusiasm (108), it may well be a most satisfactory term. Protocoproporphyria (9), however, is preferable since it emphasises a basic feature of the disorder—the enhanced faecal excretion of copro- and protoporphyrin—although it does not cover the superimposed, but temporary, pyrrol distuıbance of the acute attacks. A similar terminology emphasising the main biochemical alteration in acute intermittent porphyria and porphyria cutanea tarda symptomatica is desirable. Waldenström's (9) suggestion of pyrrolia for acute intermittent porphyria is not so facetious. The preservation of a term hallowed by long use may be achieved by combining the two: pyrrolporphyria or, more euphoniously, pyrroloporphyria. Similarly, porphyria cutanea tarda symptomatica migbt be designated urocoproporphyria. Waldenström (109) bas, in fact, recently grouped under porphyria cutanea tarda both protocoproporphyria and coproporphyria.

In endorsing Canivet & Fallot's (85) statement "Diese terminologische verwirring ist sehr bedauerlich," it is felt that the time is long overdue for a reassessment and agreement on terminology at the international level. In the meantime, in this review, the main porphyrinopathies will be listed using Waldenström's classification coupled with suggested equivalents, even at the risk of engendering further confusion!

a very disturbing finding is that 5 of 18 patients (84) did not show PBG in remission. However, ALA was not estimated. Avoidance of all barbiturates and the sulpha drugs is mandatory; pentothal sodium anaesthesia is frequently followed by catastrophic attacks.

The drugs that have been advocated are legion and the difficulties of evaluating therapy are compounded by spontaneous remissions which are often abrupt and complete. Improvement may occur despite therapy. Treatment should include prompt correction of the fluid and electrolyte depletion which is common and almost invariably the result of the preceding anorexia and vomiting. Of paramount importance is skilled nursing, if possible in a single room with reduction of all extraneous stimuli at a centre in which a respirator is available. In the event of respiratory failure full infective precautions must be applied. The severe pain may be controlled by morphine and pethidine. Chlorpromazine (155, 156) or promazine (208) has been used with great benefit. The value of the steroids is uncertain. They should be given a brief trial. In some instances, there is a rapid improvement but, undoubtedly, deterioration may occur (208) and treatment must be stopped. Chelating agents have been claimed to be beneficial (157, 158), to have induced porphyria (159), or lead to aggravation (160). Although there is no effect on ALA or PBG, the excretion of zinc is enhanced, and presumably this is desirable. We have, like others, (145) been uncertain about the benefits and feel that insufficient attention has been given to the possibility of natural remissions.

Protocoproporhyria:porphyria cutanea tarda hereditaria. (Porphyria variegata; South African genetic porphyria).—In view of the varying modes of expression this condition is best designated protocoproporphyria or porphyria variegata. It is also inherited as an autosomal dominant trait and is seen mainly in white South Africans of burgher stock (94). It is difficult to provide an overall estimate of the incidence of the condition. Although it occurs throughout South Africa, most cases have been observed in the Cape Province, especially towards the East. The incidence of about one per cent (96) claimed for the Eastern Province is probably too high. Since this was based on the screening of two selected groups (mental patients and hospital nurses) it cannot be legitimately applied to the general population. Recently, routine screening tests on all admissions to two hospitals gave an incidence of 1:223 (162). There were 8 porphyrics in 2188 males and 21 in 4272 females. Dean, who has diligently investigated the inheritance, claims that most of his porphyric patients stem from the marriage of a Dutch immigrant couple that took place at the Cape in 1688 (96). The white population in 1687 numbered only 612 (161) but, owing to very favourable conditions, this small group multiplied rapidly. No less than one million of the present three million white population hold the names of 40 original settlers (96). Thus, it is not difficult to understand why porphyria is so common among the white race. Our family studies in Cape Town are in agreement with a dominant form of inheritance and, furthermore, in the Cape Coloured people a similar

races, e.g., the Chinese (89) and the Mexican (81). A few American reports include cases in Negroes (81, 125 to 127, 151) and it is also rare in the Bantu. The author has seen 3 such cases and this is in keeping with the infrequent reports from South Africa (128 to 130) and from the rest of central and Southern Africa (131 to 134). A few cases have been reported in white South Africans (135) and in a few from North Africa (136, 137).

This form is inherited as a Mendelian dominant characteristic (9) but, in contrast to South African genetic porphyria, the skin is never involved (9). It has been recorded in identical twins (140). The clinical features of the condition are well known and, in addition to the many isolated and often indifferent reports, there have been two good reviews (9, 84). Usually urinary ALA and PBG (135, 138, 139) (sometimes PBG alone) are markedly increased in the acute attack. This usually persists after subsidence of the acute attack and is found in latent cases as well (9, 139). The faecal porphyrins are normal or slightly raised (87, 107, 135). There is still a lamentable lack of diagnostic acumen and, too often, the histories of the porphyric cases are a depressing record of erroneous diagnosis, unwarranted use of heavy sedation, and ill-considered application of electroshock therapy. The dangers of the barbiturates in this condition still do not seem to be appreciated and the use of phenobarbitone "loading" (141) is to be condemned. The unpleasant experiences of a porphyric doctor deserve to be widely read (142).

The neurological (143, 144) and psychiatric aspects (145, 146), as well as the neuropathology (143, 147, 148), have been studied extensively. The mechanisms whereby the neurologic and psychiatric symptoms are produced have been the subject of speculation for many years, but in order to explain the patchy demyelinisation, Goldberg (84) has postulated a block in the liver of the formation from porphobilinogen of a hypothetical metabolite that is essential for the nutrition of the myelin throughout the nervous system. The barbiturate-induced attack may thus be caused by inhibition in the formation of this substance in addition to depression by the drug of the oxidative mechanisms of brain tissue. While clinical evidence of hepatic dysfunction is generally lacking and structural and other change is usually absent, in fatal cases the dark colour of the liver is striking and the recognition of PBG (84, 149) in liver corroborates the findings of Schmid *et al.* (103)

The variable effects of pregnancy on the course of porphyria continue to be a matter for debate (124, 150 to 152). In most instances, deterioration is probably ascribable to the therapy and not to the pregnant state. Endocrine factors seemed to be implicated in a case (153) in which premenstrual and progesterone-induced attacks occurred, and in another where increased porphobilinogenuria accompanied ovulation (154).

The therapy of acute porphyria is still unsatisfactory. Most important is the prevention of the acute attack. Identification of latent cases amongst a patient's relatives should be done by testing for porphobilinogenuria, but

Curnow *et al.* (87), not only were the stool prophyrins normal but the skin was unaffected, thus negating the argument that the condition in South Africa is due to excessive insolation in cases of acute intermittent porphyria. There is no lack of sunshine in Perth. Treatment of the acute attack is as described in the preceding section and that for the cutaneous condition is discussed below.

Urocoproporphyria:porphyria cutanea tarda symptomatica.—Over the years many cases of porphyria (*13, 165, 170 to 172, 177*) with the purely cutaneous syndrome have been reported from many countries. In general, these cases have a later onset than the preceding types, males are much more commonly affected, a family history of the disorder is lacking, and there is an almost constant association with chronic alcoholism (164, 171, 172). The cutaneous changes are similar to those already described and full clinical and biochemical investigation of the patient and his family is necessary to distinguisb these cases from the purely cutaneous cases of protocoproporphyria. Gross sclerodermal change with mutilation may occur (13, 98). The skin histology is not diagnostic (173) and cases of porphyria with skin histology identical with lipoid proteinosis have been reported (174). In many there is clear evidence of hepatic cirrhosis, often with siderosis (114, 175, 177). Hepatomegaly is common, the so-called liver function tests are abnormal and paper electrophoresis of serum shows hypergammaglobulinaemia and sometimes mild hypoalbuminaemia (178). Hypersideraemia is frequently recorded (85, 177 to 179) but is not invariable.

Diabetes mellitus is a common association (173, 179 to 181), occurring in no less than 15 out of 36 patients (172), while Berman (180) discovered 7 male porphyrics on screening 1622 diabetics (672 males). These cases were unaware of the disorder. Pigmentation is prominent and in some cases antecedes the cutaneous fragility or occurs alone in such degree as to suggest a separate entity, called by Brugsch, melanoderma-porphyria (114, 176).

Cutaneous porphyria is common in the Bantu, not only in South Africa but also in Southern Rhodesia (182, 183), where it accounts for 2 per cent of admissions to the medical wards of Harari Hospital (184). In a South African dermatology clinic, the incidence was 1 per cent (185) as opposed to 0.2 per cent (186) in white South Africans. Tbe same erosive and bullous condition is seen but there is less photosensitivity, and bullae may occur on the palms, the tips of the fingers and subungually (98, 184). Sclerodermatous change and disfigurement, too, is more frequent. In Barnes' series (100), females outnumbered males, which is the reverse of the experience in Europe. The onset of the condition is in middle life. Darkening of the face is very common and may be the primary and indeed the sole complaint. Abnormal liver function tests and hyperglobulinaemia are frequent. Since there is no evidence of inherited disorder it is difficult to escape the conclusion that the development of this condition must in some way be related to the patient's mode of life. Most are town dwellers. The ingestion of adulterated alcohol has been postulated as a causal factor (183) as has malnutrition and "nutri-

form of inheritance may be noted (97, 98, 100). There is little doubt that the gene was originally acquired from the white race. In the Bantu, on the other hand, such cases are extremely uncommon.

The family with porphyria cutanea tarda and increased faecal porphyrins reported recently by Holti *et al.* (163) differs in no way from the South African families. Tio's (164) large family is similar although in that instance the cutaneous syndrome predominated as was the case in the families studied by Redeker (81). It would be unreasonable to propose a third dominantly inherited condition with cutaneous manifestations as the sole abnormality. In some South African families acute attacks are infrequent. Some of Watson's (104) and Brunsting's (165) cases belong to this group as, no doubt, do several isolated cases of cutaneous porphyria (166) or of acute porphyria (167). The importance of a family history and investigation of the stools of near relatives for latent cases cannot be overemphasised. Too frequently, protocols of cases lack this essential information.

The disorder usually does not manifest itself until adolescence, its peak incidence being in the third decade. Excessive cutaneous fragility and sometimes photosensitivity then become apparent. The erosive tendency and bullae formation on the sun-exposed skin surfaces are characteristic. These have been fully described (98), and do not differ materially from the condition in the symptomatic variety. It should be stressed that the cutaneous involvement may amount only to mild skin fragility (95, 98, 163) and, despite skin fragility, scarring may be insignificant. The hands may be unblemished (98). The cutaneous features may precede the acute attack by many years and may be the sole manifestation. While they usually precede, they may accompany or follow the acute attack. Pigmentation is frequent and hypertrichosis is commonly seen in women. From an analysis of 70 cases with acute attacks seen over the last 10 years (168), it is clear that abdominal, neurologic, and psychiatric syndromes are the same as those in the Swedish cases and are commoner in females. Thus, inevitably, cases have been and are still being wrongly diagnosed as cases of acute intermittent porphyria.

Characteristically, there is a marked increase in the faecal porphyrins (98, 99, 107, 169), the total commonly exceeding 500 μg./gm. dry weight with protoporphyrin contributing the major fraction. This abnormality which is present before, during, and after an acute attack varies only slightly in degree. In the acute attack, the increased excretions of ALA and PBG (98, 99, 107) can be quite as marked as in the Swedish cases. With the subsidence of the acute attacks, however, these two precursors generally return to normal. Urine uroporphyrin and coproporphyrin are usually increased especially in the acute attacks. With remission of the cutaneous syndrome the urine may become porphyrin-free. By contrast in the Swedish form, the faecal prophyrins are generally not increased (87, 107) except, possibly, during the acute stage.

In the Australian family with acute intermittent porphyria studied by

of porphyria during its use has been observed (200 to 202) and, although improvement is claimed (203, 204), the drug is of doubtful value in most cases and in many may produce a serious reaction (184, 205 to 207). Characteristically, on or about the third day there is muscle pain, backache, chills, high fever, often right upper quadrant pain, vomiting, and a massive increase in porphyrin excretion. Psychosis and coma have been noted (207). On withdrawal of the drug the fever abates and the porphyrinuria diminishes. This syndrome occurred in six out of seven Bantu with porphyria cutanea tarda treated with chloroquine (207). Similar reactioos have been noted in other Bantu patients (184). A major temporary increase in uroporphyrin and coproporphyrin in urine has been observed but there was no change in PBG or ALA, and subsequently porphyrinuria continued unchanged (208). In view of the fiodings and a possible direct "hepatotoxic effect," chloroquine therapy cannot be recommended. The beneficial effects of the chelating agents (209, 210) need to be established.

Apart from the cases attributed to alcoholic cirrhosis and "Bantu cirrhosis," Waldenström's third subgroup of "exceptional cases" was designed to cover Tio's remarkable case (74) in which remission of the cutaneous syndrome followed removal of a hepatoma. But even more fascinating is the Turkish "epidemic" which is the subject of an excellent report by Schmid (211). This is the first clear instance of widespread toxic porphyria in man. Since 1956 there have been between 3,000 to 5,000 cases in Southeastern Turkey. The familiar blistering and epidermolysis bullosa of face, hands, and feet was frequently followed by infection, and scarring and mutilation was pronounced. Marked skin pigmentation and hypertrichosis occurred. Recent weight loss, evidence of malnutrition and hepatomegaly were frequent. Abdominal and neurological complaints were absent. The urine was dark and contained ether-soluble and insoluble porphyrins but no PBG. Stool copro- and protoporphyrins were oormal in two patients and slightly increased in one (75). Findings were similar in three other cases (212). The probable relationship between this syndrome and the consumption of seed wheat treated with hexachlorobenzene-containing fungicides is supported by the production of massive uroporphyrinuria and porphobilinogenuria in rats by mixing hexachlorobenzene with the food (75). A peculiar syndrome in breast-fed babies has also been noted. Thus, the urinary and stool findings appear to correspond with the observations in other instances of symptomatic cutaneous porphyria.

Mixed porphyria.—There remains the vexed question of the identity of "mixed porphyria." Genuine combinations are possible, but most cases of mixed porphyria are probably examples of protocoproporphyria. The occurrence in a patient with symptomatic cutaneous porphyria of an abdominal disorder such as pancreatitis or an acute reaction to chloroquine therapy may be easily misinterpreted and an erroneous diagnosis made. Furthermore, in patients with cutaneous porphyria and hepatic cirrhosis the symptoms of alcoholic gastritis and peripheral neuritis may also lead to an iocorrect diagnosis of mixed porphyria. While this term may be of some

tional cirrhosis," but, in Indonesia, Tio (187) has not seen a single case of cutaneous porphyria although malnutrition and hepatic disease are fairly common.

The significance of the abnormalities in the iron metabolism is not clear. Apart from the temporary rise in serum iron during the acute attacks of protocoproporphyria, hypersideraemia is common in the Bantu cases (102, 188). Total iron binding capacity is normal (102). This resembles the picture in haemachromatosis. Hepatic siderosis is commonly associated and radio-iron studies suggest iron overload (188).

Interpretation is difficult since siderosis is common in the adult Bantu (189, 190) as many as 89 per cent of those suffering acute traumatic deaths have recognisable siderosis (191). The degree of siderosis increased with age up to 40 to 50 years and was closely correlated with the degree of cirrhosis but not with postnecrotic scarring. While hepatic siderosis may occur in the absence of cirrhosis, severe siderosis was a feature in almost all cases of cirrhosis and might be indistinguishable from haemachromatosis (192). Five per cent of adult Bantu diabetics were found to have histological features of fully developed haemachromatosis. In the Bantu, diabetes mellitus, cutaneous porphyria, hepatic siderosis, and sometimes cirrhosis occur together and a significant association has been postulated (193). Similar associations in Europeans have been previously described.

The high incidence of hepatic siderosis may be related to the very high iron intake of the Bantu. This is supported by the findings of Uys *et al.* (194) that the incidence of siderosis is significantly higher in the Bantu as opposed to the coloured who do not use iron cooking utensils habitually as the Bantu do. Hepatic siderosis is reputedly rare in Uganda (195) and in the Belgian Congo (195a) where aluminium as opposed to iron utensils are in use. It is tempting to link the lack of reports of cutaneous porphyria in Africans from these areas with the infrequency of hepatic siderosis, and to suggest that hepatic siderosis may have a significant association with porphyria, which is in line with reports of siderosis and porphyria from Europe where iron overload is uncommon.

In cases of symptomatic cutaneous porphyria, the outstanding finding is the gross uroporphyrinuria and the associated coproporphyrinuria (98, 169, 196). The claim that a characteristic fast-running, uro-type porphyrin is found on electrophoresis of urine (197) needs confirmation. Porphyrinaemia is marked, but there is no or, at most, a slight increase in ALA and PBG excretion (139, 169, 196). The faecal porphyrins are normal or slightly elevated (100 to 300 μg./gm. dried weight—mainly coproporphyrin) (98, 169) but do not approach the levels seen in protocoproporphyria (often 500 to 1000 μg./gm. and mainly protoporphyrin). Low faecal porphyrins are also characteristic of the Rhodesian cases (198).

Admission to hospital, simple dietary treatment, and withdrawal of alcohol results in marked improvement. Thus, any interpretation of the value of drug therapy must be cautious. Tio (199) has emphasised the benefit of hospitalisation alone. Chloroquine has had a great vogue, but the onset

rin Biosynthesis and Metabolism, 1955
15. Granick, S., and Mauzerall, D., *Ann. N. Y. Acad. Sci.*, 75, 115 (1958)
16. Shemin, D., and Rittenberg, D., *J. Biol. Chem.*, 159, 567 (1945)
17. Shemin, D., and Rittenberg, D., *J. Biol. Chem.*, 166, 621, 627 (1946)
18. Muir, H., and Neuberger, A., *Biochem. J.*, 47, 97 (1950)
19. Shemin, D., Russel, C. S., and Abramsky, T., *J. Biol. Chem.*, 215, 613 (1955)
20. Schulman, M. P., and Richert, D. A., *J. Biol. Chem.*, 226, 188 (1957)
21. Granick, S., *J. Biol. Chem.*, 232, 127 (1958)
22. Laver, W. G., and Neuberger, A., *Biochem. J.*, *67*, *22 p (1957)*
23. Laver, W. G., and Neuberger, A., *Biochem. J.*, 68, 17 p (1958)
24. Gibson, K. D., Laver, W. G., and Neuberger, A., *Federation Proc.*, 17, 228 (1958)
25. Gibson, K. D., *Biochem. et Biophys. Acta*, 28, 451 (1958)
26. Kiekuchi, G., Shemin, D., and Bachmann, B. J., *Biochim. et Biophys. Acta*, 28, 219 (1958)
27. Shemin, D., and Kikuchi, G., *Ann. N. Y. Acad. Sci.*, 75, 122 (1958)
28. Sano, S., Inoue, S., Tanabe, Y., Sumiya, C., and Koike, S., *Science*, 129, 275 (1959)
29. Falk, J. E., and Dresel, E. I. B., *Biochim. et Biophys. Acta*, 39, 458 (1960)
30. Westall, R. G., *Nature*, 170, 614 (1952)
31. Cookson, G. H., and Rimington, C., *Nature*, 171, 875 (1953)
32. Cookson, G. H., and Rimington, C., *Biochem. J.*, 57, 476 (1954)
33. Granick, S., and Bogorad, L., *J. Am. Chem. Soc.*, 78, 505 (1956)
34. Dresel, E. I. B., and Falk, J. E., *Nature*, 172, 1185 (1953)
35. Gibson, K. D., Neuberger, A., and Scott, J. J., *Biochem. J.*, 61, 618 (1955)
36. Schmid, R., and Shemin, D., *J. Am. Soc.*, 77, 506 (1955)
37. Granick, S., *Science*, 120, 1105 (1954)
38. Granick, S., and Mauzerall, D., *J. Biol. Chem.*, 232, 145 (1958)
39. Dresel, E. I. B., and Falk, J. E., *Biochem. J.*, 63, 80 (1956)
40. Cullity, B., and Vannotti, A., *Nature*, 185, 187 (1960)
41. Iodice, A. A., Richert, D. A., and Schulman, M. P., *Federation Proc.*, 17, 248 (1958)
42. Falk, J. E., Dresel, E. I. B., Benson, A., and Knight, B. C., *Biochem. J.*, 63, 87 (1956)
43. Booij, H. L., and Rimington, C., *Biochem. J.*, 65, 4 p (1957)
44. Lockwood, W. H., and Rimington, C., *Biochem. J.*, 67, 8 p (1957)
45. Lockwood, W. H., and Benson, A., *Biochem. J.*, 75, 372 (1960)
46. Bogorad, L., *J. Biol. Chem.*, 233, 501, 570 (1958)
47. Heath, H., and Hoare, D. S., *Biochem. J.*, 72, 14 (1959)
48. Carpenter, A. T., and Scott, J. J., *Biochem. J.*, 71, 326 (1959)
49. Heath, H., and Hoare, D. S., *Biochim. et Biophys. Acta*, 39, 167 (1960)
50. Wittenberg, J., *Nature*, 184, 876 (1959)
51. Shemin, D., Russel, C. S., and Abramsky, T., *J. Biol. Chem.*, *215*, *613* (1955)
52. Schwartz, S., *Federation Proc.*, 13, 293 (1954)
53. *Bogorad, L., Science*, 121, 878 (1955)
54. Dresel, E. I. B., and Falk, J. E., *Biochem. J.*, 63, 388 (1956)
55. Neve, R. A., Labbe, R. F., and Aldrich, R. A., *J. Am. Chem. Soc.*, 78, 691 (1956)
56. Hoare, D. S., and Heath, H., *Nature*, 181, 1592 (1958)
57. Mauzerall, D., and Granick, S., *J. Biol. Chem.*, 232, 275 (1958)
58. Rimington, C., and Booij, J., *Biochem. J.*, 65, 3 p (1957)
59. Granick, S., and Mauzerall, D., *Federation Proc.*, 17, 233 (1958)
60. Granick, S., *Federation Proc.*, 13, 219 (1954)
61. Goldberg, A., Ashenbrucker, H., Cartwright, G. E., and Wintrobe, M. M., *Blood*, 11, 821 (1956)
62. Grinstein, M., Bannerman, R. M., and Moore, C. V., *Blood*, 98, 476 (1959)
63. Schwartz, H. C., Cartwright, G. E., Smith, E., and Wintrobe, M. M., *Blood*, 14, 487 (1959)
64. Schwartz, S., and Ikeda, K., *Ciba Foundation Symposium, Porphyrin Biosynthesis and Metabolism*, 209 (1955)
65. Gajdos-Török, M., Gajdos, A., and Benard, H., *Le Sang*, 30, 459 (1959)
66. Nishude, C., and Labbe, R. F., *Biochim. et Biophys. Acta*, 31, 579 (1959)
67. Labbe, R. F., *Biochim. et Biophys. Acta*, 31, 589 (1959)
68. Goldberg, A., *Brit. J. Hematol.*, 5, 150 (1959)
69. Mazur, A., Green, S., and Carleton, A., *J. Biol. Chem.*, 235, 595 (1960)

value in describing the clinical combination of acute and cutaneous symptoms, its use to indicate a specific entity should be discontinued.

Lack of space precludes discussion of the chemical methods of investigation but Schwartz *et al.* (213) have recently published a valuable review on the determination of porphyrins in biological materials. Particular consideration is given to the general principles derived from the physiochemical properties of porphyrins. The analytical techniques described, however, are largely those in use in their laboratory. Doubt is expressed as to the validity of the determination of small amounts of PBG and ALA in normal urine, although Rimington clearly accepts the presence of these in normal urine (214). Normal values have been reported previously by several workers (71, 139, 215).

The statement that adequate data on faecal protoporphyrin are not available cannot be sustained. Admittedly, the information is meagre, but despite any defects in the method devised by Holti *et al.* (163), this is a valuable investigative procedure. Values have been reported for normal infants (216) and adults (98, 99, 163, 169), and in various porphyrin disorders (87, 98, 99, 107, 163, 164, 169).

In conclusion, it is clear that while the clinical syndromes associated with the porphyrias are often sufficiently distinctive, biochemical determination of the porphyrins and their precursors in both urine and stool is essential for their proper identification. It may be said in general that although the urine is usually adequately examined the importance of faecal porphyrin estimation has not been sufficiently widely appreciated. Furthermore, investigation of near relatives constitutes an essential part of the investigation of any porphyric patient.

While the precise defects cannot be identified as yet, there is a considerable knowledge of the main biochemical aberrations in the porphyrias. The plea is reiterated for the definition of the porphyrias in terms of major known deviations in porphyrin biosynthesis in addition to the purely descriptive terminology in current use. The hope is expressed that international agreement on terminology will not be long delayed.

LITERATURE CITED

1. Kark, R., *Med. Clin. N. Am.*, 39, 11 (1955)
2. Sunderman, F. W., Jr., and Sunderman, F. W., *Am. J. Clin. Pathol.*, 25, 1231 (1955)
3. Aldrich, R. A., Labbe, R. F., and Talman, L. E., *Am. J. Med. Sci.*, 230, 675 (1955)
4. Shemin, D., *Ciba Foundation Symposium, Porphyrin Biosynthesis and Metabolism*, 4 (1955)
5. Gray, C. H., *Lecture Sci. Basis Med.*, 4, 74 (1956)
6. Shemin, D., *Harvey Lectures, Ser. L*, 258 (1956)
7. Rimington, C., *Brit. Med. J.*, II, 189 (1956)
8. Rimington, C., *Ann. Rev. Biochem.*, 26, 561 (1957)
9. Waldenström, J., *Am. J. Med.*, 22, 758 (1958)
10. Rimington, C., *Brit. Med. Bull.*, 15, 19 (1959)
11. Gajdos, A., and Gajdos-Török, M., *Le Sang*, 30, 445 (1959)
12. Stich, W., *Klin. Wochschr.*, 37, 681 (1959)
13. Ippen, H., *Arch. klin. exptl. Dermatol.*, 208, 223, (1959)
14. *Ciba Foundation Symposium, Porphy-*

176. Brugsch, J., *Z. ges. inn. Med. u. ihre Grenzgebiete*, 11, 989 (1956)
177. Rollier, R., and Deleuze, G., *Maroc méd.*, 36, 798 (1957)
178. Filip, J., and Berman, J., *Z. ges. inn. Med. u. ihre Grenzgebiete*, 12, 476 (1957)
179. Langhof, H., *Deut. Gesundheitsw.*, 14, 1791 (1959)
180. Berman, J., *Z. ges. inn. Med. u. ihre Grenzgebiete*, 11, 186 (1956)
181. Latotzki, H., *Z. ges. inn. Med. u. ihre Grenzegebiete*, 14, 785 (1959)
182. Gelfand, M., *Central Africa Med. J.*, 1, 281 (1955)
183. Gelfand, M., and Mitchell, J. D., *Trans. Roy. Soc. Trop. Med. Hyg.*, 51, 62 (1957)
184. Gelfand, M. (Personal communication)
185. Findlay, G. H., *S. African Med. J.*, 31, 471 (1957)
186. Findlay, G. H., and Scott, F. P., *S. African Med. J.*, 34, 159 (1960)
187. Tio, T. H. (Personal communication)
188. Keeley, K. J., Bothwell, T., and Kramer, S., *Lancet*, I, 601 (1960)
189. Gillman, J. G., and Gillman, T. G., *Perspectives in Human Nutrition*, 262 (Grune & Stratton, Inc., New York, 1951)
190. Higginson, J., Gerritsen, T. H., and Walker, A. R. P., *Am. J. Pathol.*, 29, 779 (1953)
191. Bothwell, T. H., and Bradlow, B. A., *S. African J. Med. Sci.*, 24, 149 (1959)
192. Bothwell, T. H., Isaacson, C., Keeley, K. J., Seftel, H. C., and Bradlow, B. A., *Proc. 2nd Meet. Assoc. Phys. S. Afr.*, 36 (1960)
193. Keeley, K. J., *Proc. Assoc. Phys. S. Africa, 2nd Meet.*, 33 (1960)
194. Uys, C. J., van der Walt, J. J., Potgieter, G. M , and Golby, H. H., *S. African J. Lab. and Clin. Med.*, 6, 1 (1960)
195. Davies, J. N. P., and Trowell, H. C., *Brit. Med. J.*, I, 1514 (1957)
195a. Holemans, K. C. (Personal communication)
196. Barnes, H. D., *S. African Med. J.*, 33, 274 (1959)
197. Sterling, R. E., and Redeker, A. G., *Scand. J. Clin. Lab. Invest.*, 9, 407 (1957)
198. Kinnear, A. A. (Personal communication)
199. Tio, T. H., *Hautartz*, 8, 451 (1957)
200. Linden, I. H., Steffen, G. C., Newcomer, V. D., and Chapman, M., *Calif. Med.*, 81, 235 (1954)
201. Davis, M. J., and vander Ploeg, D. E., *Arch. Dermatol. and Syphilol.*, 75, 796 (1957)
202. Marsden, C. W., *Brit. J. Dermatol.*, 71, 219 (1959)
203. Colomb, M. D., *Bull. soc. franç. dermatol. Syhilig.*, 4, 420 (1957)
204. London, I. D., *Arch. Dermatol. and Syphilol.*, 75, 801 (1957)
205. Teodorescu, S., Bandaniou, A., and Gheorghiu, G., *Dermatol. Wochschr.*, 139, 445 (1959)
206. Thiers, M. M., Colomb, D., Fayolle, J., Taine, B., and Moulin, G , *Bull. soc. franç dermatol. syphilig.*, 3, 302 (1957)
207. Embree, P. (Personal communication)
208. Eales, L. (Unpublished data)
209. Peters, H. A., Eichman, P. L., and Reese, H. H., *Neurology*, 8, 621 (1958)
210. Redeker, A. G., Sterling, R. E., and Archer, B., *Arch. Internal Med.*, 104, 779 (1959)
211. Schmid, R., Report to the United States Public Health Service on a mission to Turkey, 1960
212. Barnes, H. D. (Personal communication)
213. Schwartz, S., Berg, M. H., Bossenmaler, I., and Dinsmore, H., In *Methods of Biochemical Analysis*, 8, 221 (Glick, D., Ed., Interscience Publishers, Inc., New York, 1960)
214. Rimington, C., in *Biological Aspects of Neurological Disorders*, 57 (Cumings, J. N., and Kremer, M., Eds , Blackwell Sci. Publs., Oxford, Engl., 1959)
215. Stich, W., *Klin. Wochschr.*, 36, 386 (1958)
216. Politzer, W. M., and Kessel, I., *J. Clin. Pathol.*, 11, 183 (1958)

ENDOCRINE TISSUE TRANSPLANTATION[1,2]

By John R. Brooks, M.D.[3]
Departments of Surgery, Harvard Medical School and Peter Bent Brigham Hospital, Boston, Massachusetts

Althougb it is clear that the fundamental problems of immunology must be overcome before any endocrine tissue can successfully be homotransplanted, nevertheless, it is apparent that certain endocrine tissues have weaker "histocompatibility requirements" than do skin or kidney, suggesting that these tissues exhibit variable antigenic potentialities and, therefore, may behave somewhat differently immunologically (1 to 4). Whether this is a function of antigen size (parathyroid) or possibly less reactive an antigen (ovary), or whether this has to do with the manner in which contact is made by the graft with its host (slow in the case of endocrine fragments—fast witb vascularized structures) or some other factor, is not clear.

Most endocrine organs will survive and function as grafts without the presence of a deficiency state although once established as grafts their function may well be improved by trophic stimuli. Normal nerve and lymphatic supply are probably not of vital importance to the function of a grafted endocrine gland. Although orthotopic transplantation would appear to offer the optimum chance of success (especially with pituitary), transplantation of endocrine fragments to subcutaneous or intramuscular sites and elsewhere appears to bave no overwhelming deterrent defect upon endocrine function. However, complete function may not be obtained. The reasons for this seem to be secondary to gland fragmentation or problems of vascularity and nutrition following transplantation. Endocrine tissue grafting requires minute fragmentation (1 to 2 mm.) of material in order to present the largest possible surfuce area for cross-diffusion of nutrients and metabolites. Even under optimal circumstances, a large fraction of each transplanted gland fragment undergoes central necrosis. Criteria for successful transplantation include the presence of a generous neighboring blood supply; the avoidance of pure fat as a site for implantation; the avoidance of hematomata and later scar tissue formation; the importance of using "tissue culture" techniques in the handling of tissue fragments. The onset of function of autografts (6 to 12 days) is earlier than for homografts. The site of transplantation is probably of significantly greater importance in homologous situations. Special areas such as the anterior eye chamber and brain appear to elicit a lesser immune

[1] The survey of the literature pertaining to this review was concluded in June, 1960.

[2] The following abbreviations will be used: FSH (follicle-stimulating hormone); LH (luteinizing hormone); LTH (luteotrophic hormone); TSH (thyroid-stimulating hormone).

[3] This work was supported by Grant A-1425 from the U. S. Public Health Service.

response. Embryonic and neonatal tissues appear to be better tolerated by the recipient. Successful autotransplantation of endocrine organs (thyroid, parathyroid, adrenal) by vessel-to-vessel anastomosis has also been reported.

In dealing with endocrine tissue grafts there must be clear-cut evidence of an end-organ response in order for one to report a success. To substantiate this success, the graft must be removed and normal histology found coincident with reversion of the host to the pregraft deficiency state.

A review of endocrine transplantation literature cannot but leave one in a state of confusion (67). A great body of information comes to us from work done on rats, animals notoriously impossible to evaluate genetically. Therefore, an evaluation of endocrine tissue homotransplantation in any animal and particularly the rat, at times, leads one to conclusions that seem unclear and that seem to refute heretofore classical immunological rules.

Clinically, endocrine replacement has a very special position unlike that of kidney, liver, or other organs whose replacement makes the difference between life and death. All lethal endocrine deficiencies are at least partially replaceable by medicines. For this reason, the newer methods of recipient transformation (massive bone marrow radiation and chemical disruption of antibody) have no place in endocrine tissue transplantation. Satisfactory methods for endocrine tissue replacement must be simple and risk-free.

ADRENAL

Methods of evaluating an adrenal graft have not kept pace with our present-day ability to measure circulating steroids in the blood stream and urine. Most of the literature relating to adrenal tissue transplantation has based proof of function of grafted tissue on survival of the adrenalectomized host on a salt-free diet. Removal of such a graft and subsequent demise of the animal give further corroboration to be correlated with positive histology.

Autotransplantation and Isologous Transplantation

The adrenal medulla characteristically undergoes necrosis on being transplanted. Cortical autotransplantation has been achieved by many despite the high metabolic demands of this tissue, and without the benefit of exogenous ACTH or cortisone (5). The cortical cells appear to regenerate from the region of the capsule. Such functional free fragmented autografts have been reported in rodents but only rarely in dogs (6). Using vascular anastomotic techniques, successful autografts have been reported in dogs in a few instances (7).

Homotransplantation

Successful homografts of rodent adrenal cortical tissue have been reported by several workers (8 to 11). Proof of function is not always well substantiated. The absence of accessory adrenal tissue is not always established. Occasionally, histological data are not presented. Proof of genetic dissimilarity between donor and recipient has not always been established and many

workers report failures of homotransplantation in animals without genetic compatibility (12, 13). No successful homografts in animals of higher order have been noted to this date.

Site of transplantation.—The number of successful homografts of adrenal tissue appears to increase when the anterior eye chamber (14) or cerebral cortex (15), or kidney capsule (16) are used as sites of grafting. The use of embryonic (8) or neonatal (11) tissue with or without prior cultivation (17) in recipient serum also appears to improve the chance of success. Successful growth of adrenal tissue has been reported in the hamster cheek-pouch (18).

Host or donor modification.—Successful homotransplantation of adrenal cortex following the development of immunological tolerance has been reported by a number of workers (19, 20). Others have employed tissue freezing prior to grafting with partial success. Homotransplantation of the adrenal in a diffusion chamber has been reported (4).

Human transplantation.—No long-term successful adrenal homografts have been reported in man.

OVARY

The metabolic demands of ovary are lower than those of the adrenal gland. Transplantation of its endocrine function to subcutaneous or intramuscular sites is relatively easy to accomplish when compared with transplantation of its ova-producing function. Few grafts carry ova formation through to ovulation and corpus luteum production (21). However, ovarian tissue may be transplanted orthotopically and result in fertile matings. Criteria of functional success include FSH values, vaginal cell morphology, uterine weight, cyclical menstrual periods, and fertility.

Autotransplantation and Isologous Transplantation

Autotransplantation and isologous transplantation of the fragmented ovary have been accomplished in many animals (1, 22). Subcutaneous or intramuscular grafts do produce estrogen (23) and live for an extended period of time producing cyclical menstrual periods. Occasional long-term subcutaneous grafts in rodents have been recorded (24, 25). Orthotopic autografts have been obtained (26, 27, 28).

Homotransplantation

Successful "homografts" have been reported in rats whose genetic dissimilarity was not well substantiated (25, 29). Chance genetic similarity may account for the apparently successful homografts. Delayed successful homografts have been obtained between albino and hooded rats which lasted in some cases for several months (25). A successful heterograft of ovary between the rat and the mouse was reported (30). A single instance of successful orthotopic ovarian tissue homotransplantation between dogs was also recorded (31). However, most workers have found that ovarian homografts fail to succeed when genetic dissimilarity of the paired animals exists (32 to

35). When ovarian orthotopic grafting is carried out within an isologous mouse strain, then successful fertile mating can occur. Successful homografts between newborn rats and adult recipients have been reported in sibling, intra-, and even interstrain situations (25).

Site of transplantation.—Several workers have applied the anterior eye chamber technique to ovarian transplantation with success (14, 36, 37, 38). Ovarian grafts within the spleen have resulted in tumor formation (70).

Modification of host and donor.—Transplantation of ovarian tissue following the establishment of tolerance in the recipient has been accomplished (39, 40). The production of enhancement by intraperitoneal injection of an ovarian homogenate prior to grafting has been reported (41). Fertile orthotopic ovarian grafts have been obtained by prior radiation of the recipient in intrastrain situations (42). Short-term successful takes of ovarian tissue in the rat and monkey have been obtained using the Millipore diffusion chamber to "protect" tissue from host rejection (43).

Human transplantation.—In animals of higher phylogenetic order and in humans, permanent functioning ovarian homografts have not been obtained. Functioning grafts in man within the Millipore chamber have been reported for as long as 6 months (43).

PITUITARY

The posterior pituitary is extremely difficult to transplant. The anterior pituitary autotransplanted to subcutaneous, intramuscular, and intratesticular sites functions but not infrequently loses much of its own effect on its galaxy of target organs. This loss of function is spotty and seems difficult to prognosticate. The anterior pituitary appears to function better than any other endocrine organ when it is "at home." It is possible that a trophic stimulus comes to it via the portal-hypophyseal system of vessels or by nerve conduction through the stalk (44, 45). Estimates of the success of a pituitary graft are based on proper functioning of its target organs following its transplantation.

Autotransplantation and Isologous Transplantation

Orthotopic grafts of anterior pituitary tissue have been obtained in hypophysectomized highly inbred rats (46). Body growth and secondary sex changes may occur and pregnancy results in a small percentage of cases, even in females harboring male pituitary tissue. Successful subcutaneous pituitary grafts between inbred mice have been reported with resulting maintenance of estrus (47, 48). Others report partially maintained target-organ function following pituitary isografts (23, 49). Others report no success (50). Chromophobe cells are characteristically the most hardy survivors. In general, autografts or isografts of anterior pituitary tissue when placed in abnormal sites produce variable functional results: luteinizing hormone and FSH appear less likely to be produced than LTH. Ovarian and testicular function are, there-

fore, only partially maintained. Some workers report evidence of growth maintenance; some report partial thyroid and adrenal function; others report none of either. Most of this experimental work has been carried out in mice or rats with occasional but less successful results in hamsters and rabbits. Other workers have noted resumption of gonadotrophic function when pituitary grafts were retransplanted from the kidney or temporal lobe back to the median eminence, again suggesting the need for orthotopic grafting in order to obtain proper function (45, 51, 52).

Homotransplantation

Few workers have obtained successful homografts of anterior pituitary. Those reporting successful takes obtained spermatogenesis in hypophysectomized rats but poor thyroid and adrenal function (53). Transplantation of pituitary to the spleen and sella turcica has also been accomplished in rats but function was much reduced and growth, testicular, and adrenal functions were not well maintained (54). Techniques employing prior tissue cultivation have been shown to result in homograft takes with maintenance of growth and thyroid function (55).

Site of transplantation.—Intraocular rodent homotransplants have been successful in the hands of a number of workers (54 to 59). These grafts maintain end-organ function to a variable degree. In some instances, gonadal function and fertile mating have resulted. Newborn tissue homotransplanted to the anterior chamber of rats and mice can maintain growth and testicular and ovarian function at less than normal levels (60). Similar intraocular grafts carried out in rabbits and in guinea pigs resulted in partial maintenance of pituitary gonadal function and less impressive thyroid and adrenal function (58). Others were unable to transplant successfully anterior pituitary into the rat eye chamber and no thyroid or adrenal gland function or body growth was noted. Fetal pituitary fragments placed in the anterior eye chamber in rats, however, were found to persist for two to eight months (61). Body weight and adrenal weight were well maintained, but thyroid function, only poorly. Follicle-stimulating hormone and LH production were not completely maintained (23). Sella turcica grafts appeared to function better than those in other sites (45). Partially successful grafts to the kidney capsule have been reported (51).

Transplantation of mouse and guinea pig pituitary to the atrophied testis has been shown to produce testicular hypertrophy and function (62, 63, 64). No successful canine homografts have been obtained.

Host or donor modification.—Some workers, producing tolerance in the intended recipient, have homotransplanted pituitary successfully (39). Others, using a tissue culture technique prior to homotransplantation, have been able to produce grafts that result in thyroid function and growth stimulation over a period of one to two months (65). An attempt to transplant the rat pituitary in a diffusion chamber was unsuccessful (66).

Human transplantation.—Human experimentation has been notoriously unsuccessful.

Experimental data involving transplantation of the anterior pituitary are highly variable. Autografts and homografts (following the establishment of tolerance; or to testicular and eye sites) result in much decreased to absent function. Gonadal function appears most likely to be successful. Growth, thyroid, and adrenal function are only questionably maintained.

THYROID

The metabolic demands of thyroid place it approximately midstream among the endocrine glands in the body with regard to oxygen requirements. Proof of a successful thyroid graft take is not easy to obtain and most of the experimental work is based only on histology and not on function. Simple radioactive iodine uptake by a transplanted thyroid fragment is not adequate evidence of proper thyroid function for it does not determine whether the radioactive iodine is incorporated in inorganic iodide or in the fully developed hormone tri-iodothyronine. Serum protein-bound iodine studies are of help here. Paper chromatography or red cell uptake of iodine labeled tri-iodothyronine are equally significant.

Autotransplantation and Isologous Transplantation

Autografts and isografts of mouse and rat thyroid have been performed by several workers both as thin slices and fragments and using vessel anastomosis techniques (3, 6, 68, 69). Subcutaneous transplantation has been performed in guinea pigs. Some workers, using neonatal or embryonic fragments, have successfully transplanted thyroid within a strain of albino mice. TSH therapy appears not to help.

Homotransplantation

Homografts to the usual intramuscular and subcutaneous sites do not survive a significant length of time.

Site of transplantation.—Homotransplantation between two distinct strains of rabbits using the anterior eye chamber has been reported (79). Testicular sites of thyroid transplantation have resulted in iodine^{131} uptake in guinea pig homografts (71). Thyroid fragment "takes" were obtained in dogs using a prior culture technique (72). Successful intraocular autografts and homografts have been reported in rats, mice, guinea pigs, and rabbits by some workers (14, 73, 74). Embryonic and neonatal grafts appear to improve the likelihood of a successful graft (75). Others report no success.

Host or donor modification.—Production of recipient tolerance has been carried out by several workers with resulting successful thyroid homografts (19). Using the Millipore diffusion chamber technique, successful functional thyroid autografts and homografts (76, 77) between rodents have been reported with occasional takes in dogs.

Human transplantation.—Little work has been done in man, and no permanently successful grafts have been reported. An attempt to employ the

diffusion chamber technique proved functionally unsuccessful despite persistence of thyroid follicles histologically for 12 weeks.

PARATHYROID

The parathyroid gland, because of its small size, lends itself well to minute fragmentation and transplantation. Autotransplantation in clinical practice has been substantiated. Adaptation to the hypoparathyroid state can occur both in animals and humans so that any alleged successful graft must be interpreted in this light. Newer tests of calcium and phosphorous excretion are now improving our ability to evaluate parathyroid function.

Autotransplantation and Isologous Transplantation

Successful autografts of parathyroid tissue have been reported in rodents, dogs, and human beings, using both fragment grafts and vascularized grafts (68, 78). Proof of function in these experiments is not well substantiated.

Homotransplantation

Successful parathyroid homografting in rats has been noted in a strain that routinely rejects skin grafts (2). Successful canine homografts with prior cultivation have been reported (72).

Site of transplantation.—Functional embryonic parathyroid tissue placed in the rabbit anterior eye chamber has been reported (79).

Host or donor modification.—Prior culturing techniques have produced apparently successful grafts in some instances (80, 81). The diffusion chamber technique has failed to produce long-term functioning grafts of parathyroid tissue in dogs (81a).

Human transplantation.—Techniques employing a combination of fragmented embryonic tissue cultured prior to axillary sheath implantation have been reported as successful although no histological data are obtainable (80, 81). Vascular anastomosis methods have also been reported as successful in a few cases but again lack of histology and tenuous functional data make these results open to doubt (82, 83, 84). Millipore chamber techniques have resulted in early short-lived functional takes but no successes longer than three to four months (85).

PANCREAS

The pancreas is a complicated bi-functional organ and fragmentation of its substance tends to liberate trypsin allowing autodigestion. Attempts to separate islet tissue from its exocrine portion have been carried out by many workers. This may be the *sine qua non* for pancreatic transplantation unless insulinoma tissue can be obtained.

Autotransplantation and Isologous Transplantation

Successfully functioning embryonic and adult autotransplants have been reported in the eye chamber (86) and to the abdominal wall in rodents and dogs (87, 88) and the hamster cheek pouch (89).

Human transplantation.—Human experimentation has been notoriously unsuccessful.

Experimental data involving transplantation of the anterior pituitary are highly variable. Autografts and homografts (following the establishment of tolerance; or to testicular and eye sites) result in much decreased to absent function. Gonadal function appears most likely to be successful. Growth, thyroid, and adrenal function are only questionably maintained.

THYROID

The metabolic demands of thyroid place it approximately midstream among the endocrine glands in the body with regard to oxygen requirements. Proof of a successful thyroid graft take is not easy to obtain and most of the experimental work is based only on histology and not on function. Simple radioactive iodine uptake by a transplanted thyroid fragment is not adequate evidence of proper thyroid function for it does not determine whether the radioactive iodine is incorporated in inorganic iodide or in the fully developed hormone tri-iodothyronine. Serum protein-bound iodine studies are of help here. Paper chromatography or red cell uptake of iodine labeled tri-iodothyronine are equally significant.

Autotransplantation and Isologous Transplantation

Autografts and isografts of mouse and rat thyroid have been performed by several workers both as thin slices and fragments and using vessel anastomosis techniques (3, 6, 68, 69). Subcutaneous transplantation has been performed in guinea pigs. Some workers, using neonatal or embryonic fragments, have successfully transplanted thyroid within a strain of albino mice. TSH therapy appears not to help.

Homotransplantation

Homografts to the usual intramuscular and subcutaneous sites do not survive a significant length of time.

Site of transplantation.—Homotransplantation between two distinct strains of rabbits using the anterior eye chamber has been reported (79). Testicular sites of thyroid transplantation have resulted in iodine^{131} uptake in guinea pig homografts (71). Thyroid fragment "takes" were obtained in dogs using a prior culture technique (72). Successful intraocular autografts and homografts have been reported in rats, mice, guinea pigs, and rabbits by some workers (14, 73, 74). Embryonic and neonatal grafts appear to improve the likelihood of a successful graft (75). Others report no success.

Host or donor modification.—Production of recipient tolerance has been carried out by several workers with resulting successful thyroid homografts (19). Using the Millipore diffusion chamber technique, successful functional thyroid autografts and bomografts (76, 77) between rodents have been reported with occasional takes in dogs.

Human transplantation.—Little work has been done in man, and no permanently successful grafts have been reported. An attempt to employ the

and placed in the anterior eye chamber or brain, does the functional success rate seem to improve.

The evaluation of endocrine homograft function requires proof of histology and proof of hormone output in a previous deficiency situation and then reversion back to a deficiency state on removal of the functioning graft.

Long-term successful endocrine homografts, reported in a few instances in rodents, have not yet been obtained in dogs or human patients.

LITERATURE CITED

1. Billingham, R. E., and Parkes, A. S., *Proc. Roy. Soc. (London) B*, 143, 550 (1955)
2. Russell, P. S., and Gittes, R. F., *J. Exptl. Med.*, 109, 571 (1959)
3. Woodruff, M. F. A., *J. Endocrinol.*, 11, 1 (1954)
4. Brooks, J. R., Sturgis, S. H., and Hill, G. J., 11, *Ann. N. Y. Acad. Sci.*, 87, 482 (1960)
5. Ingle, D. J., and Higgins, G. M., *Endocrinology*, 22, 458 (1938)
6. Liddle, E. B., Wittenstein, G., and Swan, H., *Surg. Forum, Proc. Am. Coll. Surgeons*, 4, 701 (1954)
7. Levy, S. E., and Blalock, A., *Ann. Surg.*, 109, 84 (1959)
8. Jones, P. F., "Preservation and Transplantation of Normal Tissues," *Ciba Foundation Symposium*, 110 (1954)
9. Darcy, D. A., *Nature*, 170, 805 (1952)
10. Wyman, L. C., and TumSuden, C., *Endocrinology*, 29, 240 (1941)
11. Higgins, G. M., and Ingle, D. J., *Anat. Record*, 70, 145 (1938)
12. Ingle, D. J., and Higgins, G. M., *Proc. Soc. Exptl. Biol. Med.*, 39, 165 (1938)
13. Everett, N. B., *Anat. Record*, 103, 335 (1949)
14. Dameron, J. T., *Proc. Soc. Exptl. Biol. Med.*, 73, 343 (1950)
15. Pomerat, C. M., Breckenridge, C. G., and Gordon, L., *Endocrinology*, 34, 60 (1944)
16. May, R. M., *Ann. N. Y. Acad. Sci.*, 64, 937 (1957)
17. Martinovitch, P. M., *J. Exptl. Zool.*, 129, 99 (1955)
18. Poor, E., *Proc. Soc. Exptl. Biol. Med.*, 97(3), 535 (1958)
19. Woodruff, M. F. A., and Sparrow, M., *J. Exptl. Physiol. & Cognate Med. Sci. (London)*, 42(2), 91 (1958)
20. Medawar, P. B., and Russell, P. S., *Immunology*, 1, 1 (1958)
21. Green, S. H., Smith, A. U., and Zuckerman, S., *J. Endocrinol.*, 13, 330 (1956)
22. Parkes, A. S., and Smith, A. U., *Acta Endocrinol.*, 17, 313 (1954)
23. Muhlbock, O., and Boot, L. M., *Ann. Endocrinol.*, 17, 338 (1956)
24. Deanesly, R., *J. Endocrinol.*, 13, 211 (1956)
25. Parkes, A. S., *J. Endocrinol.*, 13, 201 (1956)
26. Krohn, P. L., *Transplantation Bull.*, 2, 15 (1955)
27. Stevens, L. C., *Transplantation Bull.*, 4, 67 (1957)
28. Robertson, G. G., *Proc. Soc. Exptl. Biol. Med.*, 59, 30 (1945)
29. Parkes, A. S., *J. Endocrinol.*, 14, 21 (1956)
30. Saunders, J. M., *Science*, 104, 257 (1946)
31. Whitney, L. F., *Science*, 103, 654 (1946)
32. Ingram, D. L., and Krohn, P. L., *J. Endocrinol.*, 14, 110 (1956)
33. Ferguson, L., and Kirschbaum, A., *Anat. Record*, 118, 298 (1954)
34. Harris, M., and Eakin, R. M., *J. Exptl. Zool.*, 112, 131 (1949)
35. Strong, L. C., *J. Heredity*, 27, 219 (1936)
36. May, R. M., *Bull. histol. appl. physiol. et pathol. et Tech. microscop.*, 17, 51 (1940)
37. Noyes, R. W., Yamate, A. M., and Cleve, T. H., *Fertility and Sterility*, 9(2), 99 (1958)
38. Ward, J. E., Gardner, H. L., and Newton, B. I., *Am. J. Obstet. Gynecol.*, 66, 1200 (1953)
39. Martinez, C., and Smith, J. M., *Brit. J. Exptl. Pathol.*, 39(6), 574 (1958)
40. Krohn, P. L., *Bull. soc. intern. chir. (Bruxelles)*, 18(2), 182 (1959)
41. Parkes, A. S., *Transplantation Bull.*, 5(2), 45 (1958)
42. Parrott, D. M. V., and Parkes, A. S., *J. Endocrinol.*, 14, 36 (1957)
43. Castellanos, H., and Sturgis, S. H., *Obstet. Gynecol.*, 12, 603 (1958

Homotransplantation

There are few reports of successful pancreatic islet tissue homotransplantation. Homografts to the eye chamber are not successful. Successful transplantation of neonatal pancreas to the hamster cheek pouch has been reported (89).

Attempts to homotransplant the canine pancreas using vascular anastomoses have failed because of technical difficulties (88).

Human transplantation.—Clinical attempts to transplant the pancreas have been made by several workers. A successful pancreatic graft using insulinoma tissue was substantiated by histological data at the time of death of the patient (90). Others have been unable to obtain success using insulinoma tissue. A recent attempt using insulinoma tissue in Millipore chambers resulted in temporary significant clinical improvement in two total diabetic patients (85).

TESTIS

Testicular homografts and even heterografts have been reported for years in the literature. Most of these are open to scientific criticism by reason of lack of proper control. The experiments of Voronoff are included among these. Testicular autotransplantation and isografts in animals have been carried out successfully. Interstitial function was commonly obtained but rarely spermatogenesis. The presence of pituitary tissue appears to determine interstitial cell function and spermatogenesis. Several workers have reported successful takes in rodents and some of these workers have reported successful testicular homografts in rats, some to subcutaneous sites and others to the anterior eye chamber. Successful testicular takes in dogs or human beings have not as yet been reported.

SUMMARY

Functionally successful autografts of ovary, adrenal, pituitary, parathyroid, thyroid, ovary, and testis have been reported in animals of lower order (mouse, rat, guinea pig, hamster, and rabbit). Transplants to other than orthotopic sites commonly result in subnormal hormone output. There are a few scattered reports of successful autografts of adrenal, thyroid, parathyroid, pancreas, and ovary in the dog. In man, the only well-substantiated endocrine tissue autografts have been parathyroid and ovarian.

Functionally "successful" homografts of adrenal, ovary, testis, thyroid, and parathyroid have been reported in rodents. Many of these reports are based on tenuous evidence of function. Others fail to establish the genetic relationship or dissimilarity between donor and recipient. When unprotected endocrine fragments are exchanged between adult unrelated rodents and placed in subcutaneous or intramuscular sites, the likelihood of a functional success is small. When dogs or human beings represent the "experimental animal," the likelihood of a successful homograft is remote.

Only when embryonic or neonatal, or protected or cultured tissue is used

PHYSIOLOGY OF THE PLACENTA[1]

By Harry Prystowsky, M.D.

Department of Obstetrics and Gynecology, College of Medicine, University of Florida, Gainesville, Florida

In 1927, Huggett (1) reported upon his pioneer investigations which were concerned with the oxygen and carbon dioxide pressures in the maternal and fetal bloods entering the placenta of the goat. From the results of his research he inferred that the exchange of these gases takes place in that organ by diffusion. The validity of this inference appears justified, although it has not been finally established, in the sense that no one has demonstrated that the existing gradients are sufficient in themselves to provide an adequate exchange for fetal needs.

Subsequent studies have been concerned with the identification and description of those features of the placenta and the maternal and fetal bloods that might promote or limit the diffusion of oxygen and carbon dioxide. This task of identification and description is a complicated one, for the organization of the placental barrier, the vascular channels, and the characteristics of the maternal and fetal bloods differ markedly from one species to another. Accordingly, the data obtained from an investigation of one placental type are not easily applicable to a description of another. Even in the same species complications arise, for there is the time factor of gestation, i.e., there is limited applicability of data obtained at one stage in pregnancy to another stage. Another difficulty is the fact that this tissue barrier, or placenta, which separates the maternal and fetal bloods is not inert but is composed of layers of metabolically active cells that take oxygen from the current moving from the maternal toward the fetal blood and add carbon dioxide to the stream moving toward the maternal blood (2).

In an attempt to speak of the transfer of oxygen and carbon dioxide in physical terms, some have likened the placenta to a liquid block of uniform composition and thickness, having the same area on both sides. The several factors determining the rate at which a gas diffused across the placenta could then be related to the equation:

$$Q = \frac{(P_1 - P_2)(A)(k)}{D}$$

where Q is the quantity or volume transferred per unit time; P_1 and P_2, the gas tensions or pressures on its two sides; D, its thickness; A, the surface area, and k, the coefficient which represents the permeability of the barrier to the gas being studied. In respect to the placental transfer of oxygen, Q would equal the oxygen consumption of the fetus, and P_1 and P_2 would rep-

[1] The survey of the literature pertaining to this review was concluded in November, 1960.

44. Ifft, J. D., *Endocrinology*, 61(5), 595 (1957)
45. Harris, G. W., and Jacobsohn, D., *Proc. Roy. Soc. (London)*, B139, 263 (1952)
46. Greep, R. O., *Proc. Soc. Exptl. Biol. Med.*, 34, 754 (1936)
47. Wolfe, J. M., Kirtz, M. M., and Loeb, L., *Am. J. Cancer*, 38, 239 (1940)
48. Silberberg, M., Silberberg, R., and Opdyke, M., *Cancer Research*, 11, 624 (1951)
49. Scow, R. O., and Greer, M. A., *Endocrinology*, 56, 590 (1955)
50. Phelps, D., Ellison, E. T., and Burch, J. C., *Endocrinology*, 25, 227 (1939)
51. Nikitovitch-Winer, M., and Everett, J. W., *Nature*, 180, 1434 (1957)
52. Nikitovitch-Winer, M., and Everett, J. W., *Endocrinology*, 62(4), 522 (1958)
53. Courrier, R., and Colonge, A., *Compt. rend.*, 245(4), 388 (1957)
54. Cheng, C. P., Sayers, G., Goodman, L. S., and Swinyard, C. A., *Am. J. Physiol.*, 159, 426 (1949)
55. Martinovitch, P. M., *Nature*, 165, 33 (1950)
56. Greene, H. S. N., *Ann. N. Y. Acad. Sci.*, 59, 311 (1955)
57. May, R. M., *Compt. rend. soc. biol*, 124, 920 (1937)
58. Schweizer, M., and Long, M. E., *Endocrinology*, 46, 191 (1950)
59. Schweizer, M., and Long, M. E., *Endocrinology*, 47, 454 (1950)
60. Siperstein, E. R., and Greer, M. A., *J. Natl. Cancer Inst.*, 17, 569 (1956)
61. Goldberg, R. C., and Knobil, E., *Endocrinology*, 61(6), 742 (1957)
62. May, R. M., *Anat. Record*, 122, 478 (1955)
63. Petrovic, A., and Aron, M., *Compt. rend. soc. biol.*, 152(1), 144 (1958)
64. Gardner, W. U., and Hill, R. T., *Proc. Soc. Exptl. Biol. Med.*, 32, 1382 (1934)
65. Martinovitch, P. N., and Valovic, V., *Bull. Inst. Nuclear Sci. "Boris Kidrich" (Belgrade)*, 3, 131 (1953)
66. Taymor, M. L. (Personal communication)
67. Krohn, P. L., In *Transplantation of Tissues*, 401–69 (Peer, L., Ed., Williams & Wilkins Co., Baltimore, Md., 1959)
68. Dempster, W. J., and Doniach, I., *Anat. Intern. Pharmacol.*, 101, 398 (1955)
69. Kawamura, K., *J. Exptl. Med.*, 30, 45 (1919)
70. Deane, H. W., and Fawcett, D. W., *J. Natl. Cancer Inst.*, 17, 541 (1956)
71. Aron, M., Gros, C., Petrovic, A., and Gregareff, C., *Compt. rend. soc. biol.*, 149, 407 (1955)
72. Stone, H., Owings, J., and Gey, *Surg. Gynecol. Obstet.*, 60, 390 (1935)
73. Dameron, J. T., *Surg. Forum, Proc. Am. Coll. Surgeons*, 3, 681 (1953)
74. Woodruff, M. F. A., "Evidence for Adaptation in Homographs of Normal Tissue," in *Biological Problems of Grafting*—A Symposium (Blackwell Sci. Publs., Oxford, Engl., 1959)
75. Salmon, T. N., and Severinghouse, A. E., *Proc. Soc. Exptl. Biol. Med.*, 34, 251 (1936)
76. Duthie, R. B., Merwin, R. M., and Wolff, J., *Exptl. Cell Research*, 16, 565 (1959)
77. Brooks, J. R., Hill, G. J., II, de'Scoville, A., Priario, J., Crocker, D., and Selenkow, H. A., *Endocrinology*, 66, 392 (1960)
78. Jordan, G. L., Foster, R. P., and Gyorkey, F., *Transplantation Bull.*, 5, 392 (1958)
79. May, R. M., *Arch. Anat. Histol. Embryol.*, 21, 31 (1936)
80. Kooreman, P. J., and Gaillard, P. J., *Arch. chir. Neerlandium*, 2, 326 (1950)
81. Kempe, C. H., and Jawetz, M. J. M., *Transplantation Bull.*, 5, 155 (1958)
81a. Wilson, R. E., Zollinger, R., Mahan, J., and Brooks, J. R., *Surg. Forum, Proc. Am. Coll. Surgeons*, 10, 94 (1959)
82. Sterling, J. A., *Transplantation Bull.*, 5, 50 (1958)
83. Jordan, G. L., Foster, R. P., and Curd, G. W., Jr., *Transplantation Bull.*, 5, 49 (1958)
84. Watkins, E., Jr., Haynes, L. L., and Adams, H. D., *Pediatrics*, 21(6), 974 (1958)
85. Brooks, J. R., and Hill, G. J., II, *Am. J. Surg.*, 99, 588 (1960)
86. Browning, H., and Resnick, P., *Yale J. Biol. Med.*, 24, 141 (1951)
87. Ivy, A. C., and Farrell, J. I., *Am. J. Physiol.*, 77, 474 (1926)
88. Brooks, J. R., and Gifford, G. H., *Transplantation Bull.*, 6, 100 (1959)
89. House, E. L., Burton, C., Cooper, H., and Anderson, E., *Endocrinology*, 63(3), 389 (1958)
90. Gaillard, P. J. (Personal communication)

If one is to compare the functional capacities of placentas as Krogh did in regard to lungs, it is necessary to know the rate of oxygen consumption of the fetus, its weight, and the difference in the oxygen pressures of the maternal and fetal bloods that promotes the transfer. To my knowledge no one has measured the rate of oxygen consumption of the fetus *in utero* under circumstances in which it was known that the fetus was meeting all its metabolic requirements by oxygen derived from the fetal blood and not developing an oxygen debt. This is an important point, for the evidence now at hand indicates that when the mother is unanesthetized and at rest, the tissues of the fetus are not engaged in anaerobic metabolism. On the other hand, interference with her level of oxygenation or with the uterine circulation by light anesthesia may cause them to do so (5).

There are some estimates based on indirect methods of the rate of oxygen consumption, and they have been obtained on fetuses of the guinea pig (6), rabbit (7), goat (8), and sheep (9, 10) at, or near, term. The values reported indicate that the rate at that stage of pregnancy is approximately that of the adult of the same species, i.e., about 8 ml. per kg. per min. for the guinea pig and rabbit, 5 for the sheep, and 6 for the goat. In addition, there are data to indicate that in the sheep (11, 12) the rate may be much higher earlier in gestation, i.e., approximately 10 to 12 ml. per kg. per min. between the 95th and 110th days.

The estimation of the oxygen tensions in the region where the exchange between maternal and fetal blood takes place is no less difficult than the estimation of the fetal oxygen consumption; the results at hand are not entirely satisfactory. If one makes the assumption that only insignificant amounts of maternal and fetal blood pass through the placenta via channels that exclude the transfer of oxygen between them, the oxygen pressure in the blood at the ends of the maternal and fetal capillaries on the two sides of the barrier will be the same as those in the principal vessels leading to and from their respective capillary nets. In other words, the oxygen tension at the arterial end of the maternal capillary will be the same as that of the blood in the uterine artery, and at the venous end the pressure will be the same as it is in a uterine vein. On the fetal side the pressures will be equivalent to those of the bloods of the umbilical artery and vein, respectively.

In most studies the oxygen tensions in samples drawn from the uterine and umbilical vessels have been estimated by relating their percentage saturation to an appropriate oxygen dissociation curve. In estimating oxygen pressure the effect of the pH of the blood on its oxygen content or on its percentage saturation is neglected, and the saturation is referred to a curve prepared at known pH or CO_2 pressures. More recently, however, oxygen pressure in such samples has been measured directly with the dropping mercury electrode. Some data have also been obtained recently in which the pH of the blood samples drawn was determined as well as the percentage

resent the pressure values for oxygen in the maternal and fetal bloods, respectively.

The usefulness of this equation for a description of placental gas exchange is very limited and the same holds true for the lung, for direct measurements of A, D, and k, are not available for either organ. And they are not likely to become available soon because of technical difficulties. Despite the absence of these data on dimensions, however, meaningful comparisons have been made of the functional capacities of placentas of a given species taken at different stages in gestation, and of placentas of different species, just as such comparisons have been made of the functional capacities of the lung.

As will be recalled, in his approach to the study of the lung Krogh (3) introduced the concept of the "diffusion constant" and defined it as the volume of oxygen in milliliters which will pass through the pulmonary epithelium per minute when the difference in oxygen pressure on the two sides of the membrane is 1 mm. Hg. Investigators in placental physiology have followed Krogh's approach and have been concerned with the difference in pressure required to effect the transfer of oxygen for, in essence, this tension difference may be looked upon as an index of the permeability of the placenta. The greater the pressure difference required to effect a given transfer rate, the lower the permeability and vice versa. Or, stated in another way, the greater the quantity of oxygen transferred per millimeter difference in pressure on the two sides of the barrier, the greater the permeability.

Krogh proceeded even further and attempted to estimate the over-all or functional permeability in terms of the requirements of the organism served by the lung. To permit comparisons of permeability in terms of the requirements of the organism, he divided the diffusion constant by the surface area of the body (an index of basal metabolic rate) in order to obtain the quantity of oxygen transferred across the lung per minute per millimeter pressure difference per square meter of body surface. Although surface area is the dimension of the body that is most readily determined and that shows the closest correlation with metabolic rate, the correlation between body weight is sufficiently close so that there would appear to be no significant error introduced if the weight of the fetus were substituted for its surface area, when comparing the diffusion rates across placentas of different ages and types. If this substitution is justified, then a comparison can be made of placentas as organs for gas exchange on the basis of the quantity of oxygen that is transferred across them per minute per millimeter difference in gas pressure in the maternal and fetal bloods per kilogram of fetal tissue. It is important to stress the fact that the rate of transfer should be related to fetal weight and not placental weight, for only the fetal tissues are supplied with oxygen that actually reaches the fetal blood, and, furthermore, there is apparently no useful correlation between the dimensions of the placenta as related to diffusion and its weight (4).

sumptions and given the following data: the oxygen dissociation curves of the fetal and maternal bloods, their oxygen capacities, and the percentage saturation of blood in the uterine artery, uterine vein, umbilical vein, and umbilical artery. The assumptions are: (*a*) that each stream moves through vessels of constant caliber at a uniform velocity; (*b*) that the permeability to oxygen of the placental capillaries and the intervening tissues is uniform throughout their length; (*c*) that the volume of oxygen diffusing per unit of time from the blood in one regioo of the maternal capillaries to the blood in a region of the fetal capillaries is proportional to the difference in oxygen pressure between the two regions (28, 29).

At the present time, estimates have been made of the oxygen pressure gradient across the placenta for three species: sheep (28), goat (15, 30), and rabbit (18). The results indicate gradients of oxygen tension of the order of 40, 37, and 10 mm. Hg, respectively. Estimates of the oxygen pressure gradient across the placental barrier in man have been made by comparing the oxygen pressure in blood drawn from the intervillous space with the pressure in bloods drawn from the umbilical artery and vein, respectively, at the time of cesarean section and vaginal delivery. The gradient of oxygeo tension appears to be of the order of 24 mm. Hg (19, 20).

In man it appears that the placenta is a less efficient organ for the oxygenation of blood than is the lung. A higher oxygen pressure gradient is required to provide the oxygen requirements of the individual when the gas must cross the placenta than when it crosses a lung. Stated in another way, the oxygen requirements of the mammalian fetus at the end of term when expressed in cubic centimeters per kilogram of body weight per minute are of the same order os those of the adult. Yet, a higher pressure difference is required to meet the fetal needs across the placenta than is necessary in the case of the lung of the adult. In man, a difference io oxygen pressure across the lungs of 10 mm. Hg is sufficient to provide his requirements at rest, whereas a pressure difference of twice that order is required to provide the normal oxygen requirements of the fetus *in utero.*

The factors that are responsible for this steep gradient have not been identified, but evidence is accumulating which indicates that it is the result of mechanisms that can be altered quite rapidly and is not determined by the structure of the placenta *per se,* as had been suggested earlier (31). The gradient appears to be adjusted and maintained by the fetus to provide it with an adequate oxygen supply despite wide variations in the tension of that gas in the maternal blood reaching the uterus. The evidence for this viewpoint comes from three sources: (*a*) the data demonstrating that in ewes that live and reproduce at high altitudes, at which the oxygen pressure in the arterial blood is about 47 mm. Hg, the oxygen tension in the fetal blood and the oxygen consumption of the uterus and its contents in cubic centimeters per kilogram per minute are comparable to sea level values (32).

saturation, and the results permit a more accurate estimate of the oxygen tension in those circumstances in which the effects of pH on the oxygen dissociation curve of the blood tested are known.

By these methods estimates have been made of the oxygen tension in bloods from the uterine artery, uterine vein, umbilical artery, and umbilical vein at one or more stages in gestation in the sheep (13, 14), goat (1, 15), cow (16, 17), and rabbit (18), when the vessels are exposed at cesarean section and at the time of vaginal delivery. In a number of cases these values have been compared with the oxygen pressure of blood drawn from the intervillous space of the human (19, 20). In other cases the comparison has been made with the oxygen tension in blood from a superficial maternal vein or an artery (21, 22, 23).

Although these data may be of value for a general comparison of the oxygen tensions in the bloods in the uterine and umbilical circulations, they cannot be used directly for the estimation of the gradient across the placental barrier. For this estimate one must know the single pressure values which would result in the same transfer as the varied oxygen tensions that do exist between the arterial and venous ends of the uterine and umbilical circulations, respectively, and the pattern of flow in these two circuits relative to one another.

In many placentas the maternal and fetal bloods (24, 25, 26) tend to move in opposite directions through more or less parallel capillary cords. Hence, blood leaving the placenta via the fetal capillaries is last opposed by arterialized maternal blood entering that organ. The placental capillary nets and the pattern in which they are perfused in essence form a countercurrent exchange system. Mossman (27) was the first to point out that when the fetal and maternal bloods move in the same direction, the maximum pressure and saturation in the fetal blood will be determined by an equilibrium with the venous blood leaving the placental capillaries; whereas on the countercurrent principle, the theoretical maximum would be attained when the fetal blood leaving the placental capillaries was in equilibrium with the maternal arterial blood entering the placenta. Accordingly, the countercurrent arrangement in the placenta serves three functions: (*a*) to permit the concentration of a substance in the blood into which it diffuses to approach the maximum concentration in the blood giving it up; (*b*) to minimize the effect of the quantity that moves from one blood to the other at one point in the exchange system, on the concentration difference available to promote the movement; (*c*) to maintain a more uniform difference in the concentration of the diffusible substance between the two sides along the length of the parallel capillary nets.

Where the fetal and maternal bloods are known to pass through more or less parallel capillary cords in opposite directions, the gradient of oxygen pressure across the placental barrier can be estimated, granted certain as-

the placenta. It has been suggested that since a relatively large pH gradient is maintained across the placenta, the Bohr effect would tend to superimpose the fetal oxygen dissociation curve upon that of the mother *in vivo*. While this circumstance in itself would not abolish the oxygen pressure gradient across the placenta, it would certainly not facilitate it. Recent data (41) question the above-mentioned concept and indicate that the maternal and fetal oxygen dissociation curves *in vivo* are not normally superimposed. Furthermore, the suggestion regarding superimposition of the maternal and fetal oxygen dissociation curves *in vivo* is based on the assumption that the Bohr effect on maternal and fetal blood is identical. This is not the case for the goat (44), sheep (45), llama (46), and man (47).

DERMATOLOGY: THE ECCRINE SWEAT GLANDS[1]

By W. C. Lobitz, Jr.[2] M.D.[3]

Division of Dermatology, University of Oregon Medical School, Portland, Oregon

AND

R. L. Dobson, M.D.[3]

Division of Dermatology, University of North Carolina, Chapel Hill, North Carolina

Although much is known about the human eccrine sweat glands as a result of extensive studies carried out during the past half century, surprisingly little can be stated concerning the function of these organs without fear of contradiction. This situation has arisen for three major reasons: (*a*) It is now known that during active function the cellular structure of the eccrine sweat gland is undergoing significant alterations. (*b*) The function of the sweat gland is, in great part, dependent on the state of both the external and internal environment. (Careful control of both of these variables is essential to an experimental situation from which interpretable information is to be derived.) (*c*) Interpretation of the function of the sweat gland is considerably influenced by the method of sweat collection, the area from which the sweat is collected and the method employed to produce sweating.

This review will not deal with specific diseases of the eccrine sweat gland. Nor will it be concerned with the alterations in structure and function of the sweat apparatus that occur when the skin itself is altered by disease. We will not emphasize environmental physiology and the problems of acclimatization. The reader is referred to a most excellent and comprehensive review by Weiner & Hellmann (53) on the total eccrine and apocrine sweat gland story. The interested reader, too, should refer to the recent book edited by Yoshimura, Ogata & Itoh on climatic physiology (54).

In this review it is our hope to integrate, as much as possible, the many aspects now known of eccrine sweat gland structure and function in health and associated with non-cutaneous medical disease. In this way perhaps the interpretations of isolated findings on this subject will be made easier. In addition, we will take opportunities, too. to emphasize the many areas in which further investigative studies may be especially fruitful.

On the basis of location, two types of eccrine glands can be differentiated: those on the palms and soles which respond primarily to mental work and emotional stimuli, and those in the remainder of the skin which act mainly in a thermoregulatory role. All eccrine sweat glands are innervated by sympathetic fibers, but the secretion is induced by cholinergic agents. The nerve

[1] The survey of the literature pertaining to this review was concluded in June, 1960.

Preparation of this review was supported in part by the United States Public Health Service, NIAID Grant E-3102[2], NIAMD Grant A-1908(R1)[3] and in part by the Office of the Surgeon General (Contract No. DA-49-007-MD-977).[3]

LITERATURE CITED

1. Huggett, A. St. G., *J. Physiol.*, 62, 373 (1927)
2. Kustner, H., and Siedentopf, H., *Arch. F. Gynäkol.*, 138, 131 (1929)
3. Krogh, M., *J. Physiol.*, 49, 271 (1915)
4. Barcroft, J., and Kennedy, J. A., *J. Physiol.*, 95, 173 (1939)
5. Huckabee, W. E., Metcalfe, J., Prystowsky, H., and Barron, D. H. *Federation Proc.*, 16, 102 (1957)
6. Bohr, C., *Skand. Arch. Physiol.*, 10, 413 (1900)
7. Barron, D. H., and Meschia, G., *Cold Spring Harbor Symposia Quant. Biol.*, 19, 93 (1954)
8. Barcroft, J., Flexner, L. B., and McClurkin, T., *J. Physiol.*, 83, 498 (1934)
9. Barcroft, J., Kennedy, J. A., and Mason, M. F., *J. Physiol.*, 95, 269 (1939)
10. Dawes, G. S., Mott, J. C., and Widdecomb, J. G., *J. Physiol.*, 126, 563 (1954)
11. Barcroft, J., and Elsden, S. R., *J. Physiol.*, 105, 25 (1946)
12. Carlyle, A., *J. Physiol.*, 107, 355 (1947)
13. Barron, D. H., *Yale J. Biol. Med.*, 19, 23 (1946)
14. Barcroft, J., *Researches on Prenatal Life*, 292 (Charles C Thomas, Publ., Springfield, Ill., 1947)
15. Prystowsky, H., Meschia, G., and Barron, D. H., *Yale J. Biol. Med.*, 32, 441 (1960)
16. Roos, J., and Romijn, C., *J. Physiol.*, 92, 249 (1938)
17. Roos, J., and Romijn, C., *Proc. Koninkl. Ned. Akad. Wetenschap.*, 43, 1 (1940)
18. Barron, D. H., and Battaglia, F. C., *Yale J. Biol. Med.*, 28, 197 (1955)
19. Prystowsky, H., *Bull. Johns Hopkins Hosp.*, 101, 48 (1957)
20. Prystowsky, H., Hellegers, A. E., and Bruns, P. B., *Surg. Gynecol. Obstet.*, 110, 495 (1960)
21. Eastman, N. J., *Bull. Johns Hopkins Hosp.*, 37, 221 (1930)
22. Hazelhorst, G., and Stromberger, K., *Z. Geburtshilfe u. Gynäkol.*, 100, 43 (1931)
23. Beer, R., Bartels, H., and Raczkowski, H. A., *Arch. ges. Physiol.*, 260, 306 (1955)
24. Tafani, A., *Arch. ital. biol.*, 8, 49 (1887)
25. Mossman, H. W., *Carnegie Inst. Wash. Contrib. Embryol.*, No. 26, 129 (1937)
26. Barcroft, J., and Barron, D. H., *Anat. Record*, 94, 569 (1946)
27. Mossman, H. W., *Am. J. Anat.*, 37, 433 (1926)
28. Barron, D. H., and Alexander, G., *Yale J. Biol. Med.*, 25, 61 (1952)
29. Lamport, H., *Yale J. Biol. Med.*, 27, 26 (1954)
30. Prystowsky, H., Barron, D. H., Metcalfe, J., and Huckabee, W. E., *Federation Proc.*, 16, 102 (1957)
31. Barron, D. H., *Études neo-natales*, 1, 3 (1952)
32. Metcalfe, J., Meschia, G., Hellegers, A. E., Prystowsky, H., Huckabee, W. E., and Barron, D. H., *Federation Proc.*, 18, 104 (1959)
33. Kaiser, I. H., Cummings, J. M., Reynolds, S. R. M., and Marbarger, J. P., *J. Appl. Physiol.*, 13, 171 (1958)
34. Prystowsky, H., Barron, D. H., Metcalfe, J., and Huckabee, W. E. (Unpublished data)
35. Keys, A. B., *J. Physiol.*, 80, 491 (1934)
36. Barron, D. H., *Federation Proc.*, 8, 8 (1949)
37. Roos, J., and Romijn, C., *Proc. Koninkl. Ned. Akad. Wetenschap.*, 43, 21 (1940)
38. Young, I. M., *Am. J. Physiol.*, 170, 434 (1952)
39. Barron, D. H., and Meschia, G., *Yale J. Biol. Med.*, 29, 480 (1957)
40. Kaiser, I. H., and Cummings, J. M., *J. Appl. Physiol.*, 10, 484 (1957)
41. Prystowsky, H., Hellegers, A. E., and Bruns, P. B., *Am. J. Obstet. Gynecol.* (In press)
42. Kaiser, I. H., and Goodlin, R. C., *Pediatrics*, 22, 1097 (1958)
43. James, L. S., Weisbrot, I. M., Prince, C. E., Holaday, D. A., and Apgar, V., *J. Pediat.*, 52, 379 (1958)
44. Hellegers, A. E., Meschia, G., Prystowsky, H., Wolkoff, A. S., and Barron, D. H., *Quart. J. Exptl. Physiol.*, 44, 215 (1959)
45. Meschia, G., Prystowsky, H., Hellegers, A. E., Huckabee, W. E., Metcalfe, J., and Barron, D. H., *Quart. J. Exptl. Physiol.* (In press)
46. Meschia, G., Prystowsky, H., Hellegers, A. E., Huckabee, W. E., Metcalfe, J., and Barron, D. H., *Quart. J. Exptl. Physiol.*, 45, 284 (1960)
47. Prystowsky, H., Hellegers, A. E., and Bruns, P. B. (Unpublished data)

The concentration of urea is enormously variable, with a range of 3 to 200 mg. per 100 ml.

It is difficult to appraise the meaning of the so-called normal concentration of sodium chloride in sweat. So many variables enter into this situation that to speak of a normal sweat sodium or chloride is probably as meaningless as to refer to a normal urinary sodium or chloride. It may, however, be stated with reasonable certainty that the sweat sodium or chloride concentration seldom exceeds 80 m.eq./l. It is usually true, also, that the concentration of sodium exceeds that of chloride by about 10 per cent. This is by no means a constant finding, and the ratio can easily be reversed by the administration of large amounts of sodium chloride to a normal individual (11).

Although it seems likely that active reabsorption occurs in the sweat duct, the evidence for this is mainly inferential and circumstantial. Ackermann (12) was probably the first to suggest it. Later, Lobitz & Mason (13) found that many constituents of sweat were more concentrated at low rates of sweating. They suggested that at these slow rates there was greater opportunity for the ducts to reabsorb water.

Schwartz, Thaysen & Dole (14) studied the excretion of urea in eccrine sweat. Their findings implied that urea is carried into the duct in some precursor solution and then concentrated by the absorption of a constant fraction of water. It has been shown, however, that the urea concentration of sweat may be less than that of plasma, especially at high rates of sweating (11). This finding is difficult to reconcile with the hypothesis of the previous authors.

More recently, Schwartz & Thaysen (15) studied the excretion of sodium and potassium in sweat. Sweat sodium concentration was hypotonic to plasma, ranging from 6 to 85 m.eq./l. Potassium, on the other hand, was invariably hypertonic. By comparing the rate of excretion of sodium and potassium with the rate of flow of sweat, they concluded that sodium, but not potassium, is reabsorbed by the duct by a process of limited capacity. Similar results were obtained by Bulmer & Forwell (16).

Although the urea concentration of sweat is inversely proportional to the rate of sweating (11), the concentration of sodium tends to be high at both high and low rates of sweating. The lowest concentrations of sodium were found at moderate rates of sweating. It is thus apparent that simple reabsorption of water is not a suitable explanation for the regulation of the final composition of eccrine sweat.

Recently, two different and novel approaches have been taken to investigate the problem of reabsorption in the sweat duct. Thompson (17) found that in autogenous free grafts of human dermis buried subcutaneously for periods extending to five years, the eccrine sweat glands with their attached ducts survived. The ducts formed elongated blind sacs which did not reach the surface of the skin. After pilocarpine stimulation of the grafts, there was depletion of glycogen from both the secretory coil and duct. Since depletion of glycogen from the secretory coil is always considered to be asso-

fibers which surround the secretory portion of the eccrine gland have a rich content of acetylcholinesterase (1). Eccrine sweat can also be elicited about 80 per cent of the time by the local injection of epinephrine (2).

Anatomically, the eccrine gland consists of a simple, tubular duct and a coiled secretory portion. The duct itself is composed of a short coiled portion, a long and relatively straight portion extending from the coil through the dermis to the epidermis, and the intraepidermal portion lying within the "epidermal eccrine sweat duct unit" (3, 4, 5). The microanatomy of the eccrine sweat duct (exclusive of its epidermal portion) consists of an inner luminal cell and an outer basal cell, both of which are metabolically active and rich in glycogen. The secretory coil has one cell layer surrounded by myo-epithelial cells and a basement membrane. In the single layer of secretory cells there are two distinct cell types: a large cell without secretory granules but rich in glycogen, and a small cell laden with metachromatic, Schiff-reactive granules containing relatively little glycogen. Conventionally, these cells are referred to as the "large, pale cell" and the "small, dark cell," respectively (because of their appearance when stained with toluidine blue buffered at pH5). On the basis of recent electron microscopic studies (6), it has been suggested that the small dark cell represents the presecretory phase of the large pale cell. Another suggestion (7) has been that the large pale cells are concerned with actual sweat secretion and that the small dark cell secretes only a mucoid material. We would tend to agree with the latter statement, since a diminution in the number of large pale cells occurs as a result of repeated consecutive episodes of profuse sweating (8) In general, the histologic appearance of the eccrine gland is rather uniform, but marked individual variations may be observed (9).

The factors controlling the normal composition of sweat have not been extensively studied and are as yet poorly understood. Most authors agree that a "precursor fluid" derived from plasma is secreted with modifications by the secretory coil, and that the ultimate composition of the sweat is dependent upon the activity of the duct. At present, however, nothing is known about the role of the secretory coil in the formation of "precursor fluid." The secretory coil apparently is impermeable to plasma proteins and glucose, but various other constituents of plasma pass through freely. These include water, sodium, potassium, chloride, urea, creatine, creatinine, lactate, and phosphate. Small amounts of mucoprotein are present in sweat, but these are probably derived from the cells of the secretory coil themselves (10). The appreciable lactate content of sweat is, in part, attributed to metabolic activity of the gland and duct, since there is considerable carbohydrate metabolism in both during active sweating. Sodium and chloride are usually hypotonic but may be hypertonic under the stimulus of a high salt load, especially at low rates of sweating (11). Potassium, on the other hand, is almost invariahly hypertonic. Some of the potassium may be caused by the metabolic breakdown of glycogen during secretion, and for this reason can perhaps be eliminated from a discussion of the regulation of the composition of sweat.

to define "fatigue" as a decrease in the rate of sweating per gland. Sargent (24) has suggested the name "hidromeiosis" for this latter phenomenon.

Despite an enormous amount of work done by many investigators, the chemical composition of sweat and its regulation are far from being established and understood. This subject was thoroughly reviewed by Robinson & Robinson (21) in 1954. It is generally agreed that sweat contains about the same constituents as urine. Sodium and chloride are usually hypotonic to plasma while potassium and urea are hypertonic. The coocentrations of all constituents vary widely and are dependent on many local and systemic factors such as rate of sweating, skin temperature, collection area, and method of collection. It has been generally accepted, for example, that the chloride concentration of sweat is proportional to the rate of sweating. This applies when short collection periods are utilized, during the first few hours after the induction of sweating. If sweating continues, a phase is reached when chloride concentratioo and sweat rate are approximately constant. However, after 4 to 6 hr. of thermal sweating, as the rate of sweating declines, chloride concentration increases so that under these circumstances sweat rate and chloride concentration are inversely related (11). Skin temperature also influences the composition of sweat (25), and the sodium chloride concentration tends to be lower on cooled portions of the body and higher on heated areas. The electrolyte concentration of sweat will also vary depending on the area of the body from which it has been collected. This is especially apparent with palmar sweat, which tends to be considerably more concentrated than that from other areas of the body at low rates of sweating (26). Furthermore, it has been shown that collecting sweat under a vapor-impermeable barrier will result in a decreased rate of potassium excretion and an increase in that of creatinine (27). Previous exposure to a hot environment is also of considerable significance. The fully heat-acclimatized individual, for example, may excrete sweat with a sodium concentration of less than 10 m.eq./l., whereas an unacclimatized subject can have sweat sodium concentrations hypertonic to plasma especially on a diet high in sodium (11).

Perhaps the major reason for these variations is a factor which only in recent years has come to be appreciated. The anatomy of the sweat glaod is not static but undergoes wide cytologic changes as a result of sweating. In 1935, Yuyama observed a loss of glycogen from the secretory cells of the eccrine sweat gland after stimulation with acetylcholine (28). This finding wos later confirmed by Shelley & Mescon (29). In more recent years, the subject has been extensively studied by Dobson *et al.* (8, 30, 31). It was found initially that as a result of a single 6-hr. episode of profuse sweating, marked morphologic changes occurred in the cells of the eccrine sweat gland. These changes consisted of loss of glycogen from both the secretory coil ood duct, vacuolization and atrophy of the large pale cells, and depletion of Schiff-positive, diastase-resistant (SPDR) material from the small dark cells of the secretory coil. Recovery from these effects of profuse sweating has an orderly progression, the major features of which include a return of glycogen

ciated with active sweating, and since the duct did not dilate, it must be assumed that reabsorption of sweat must have occurred. The problem was approached in a different manner by Lloyd (18), who argued that, assumiog the ducts of resting sweat glands to be empty, on stimulation of the secretory coil sweat formation would begin. A phase of duct filling would antecede sweat emergence at the skin surface. When stimulation was stopped, sweat formation would cease but the resting condition would be regained only when the ducts again emptied through reabsorption. Using the paw of a cat and stimulating the secretomotor nerve, he showed that the duration of the rest periods between stimulation was directly proportional to the latent period of emergence of sweat on the surface.

The microanatomy of the sweat duct also suggests that it plays ao active role in the regulation of the composition of sweat. Its cells are rich in glycogen, mitchondria, ribonucleic acid, and succinic dehydrogenase, which implies an active metabolic function (1). Lobitz, Holyoke & Brophy (5) have presented direct histochemical evidence of sweat duct activity. They showed that in the two-cell layered duct, only the basal cells have the power of regeneration (4). Since the luminal cells are unable to undergo mitotic division, any histochemical activity that can be demonstrated in them must be concerned with metabolic function and not reproduction. After stimulation of the sweat gland with local injections of acetylcholine, these authors found that the linear band of glycogen in tbe luminal cell of the duct disappears and is replaced by a dense band of succinic dehydrogenase activity.

Thus, the weight of evidence speaks strongly for active participation by the sweat duct in the ultimate regulation of the composition of sweat. The mechanism of the process, however, is still conjectural and much remains to be done before a reasonable explanation of "tubular" function in the eccrine sweat gland can be advanced.

With constant thermal stimulation, or with repeated injections of cholinergic drugs such as methacholine, a marked decrease in the output of sweat per unit area is noted after 4 to 6 hr. (19, 20). This bas been referred to as sweat gland fatigue. In thermally stimulated glands, this fatigue is associated witb an increase in the concentration of sodium in tbe sweat (21). In glands wbich become fatigued after repeated intracutaneous injections of methacholine, however, the concentration of sodium in the sweat decreases (22). This paradoxical situation may recently have been clarified by Collins, Sargent & Weiner (23) who found that repeated injections of acetylcholine or methacholine in high concentrations (200 to 400 μgm/0.05 ml.) produced a progressive reduction in the number of functioning sweat glands. Their evidence suggested that a neurohumoral block rather than "fatigue" was responsible for the depression of sweating. If conventional methods of collecting sweat are employed, lowered output could be interpreted as "fatigue" in terms of the amount of sweat produced per unit area. The critical question is whether the term "sweat gland fatigue" is properly applied to a decrease in the number of functioning glands per unit area. It seems more reasooable

adrenal gland, it would be of interest to know what the effect of aldosterone per se might be on the structure of the sweat gland.

In addition to Cushing's syndrome, the chemical composition of sweat has been found to be altered in a variety of systemic diseases. Conn has shown that the chloride content of sweat is elevated in adrenal insufficiency (34). After the administration of adrenal corticosteroids, the chloride resumed a normal concentration. The responsiveness of the sweat glands to adrenal cortical activity has led Conn *et al.* (35) and Grønbaek (36) to advocate the determination of sweat electrolytes as an index of adrenal cortical function. Moseley and his co-workers (37) have reported that the concentration of sodium in the sweat is decreased in hyperthyroidism and increased in hypothyroidism. These authors suggested that changes in adrenal cortical activity in thyroid disease were responsible for their findings. In hypothyroidism it has recently been shown that the large pale cells of the secretory coil contain large granules of an amorphous mucoid material which could conceivably influence the function of the gland (38). It would be most interesting to know what changes such an abnormal gland would undergo while under the influence of profuse sweating. In hepatic cirrhosis, according to Eisenmenger *et al.* (39), there is a decrease in the concentration of sodium in the sweat.

An interesting alteration in sweating in nephrosis has been reported by Warming-Larsen & Wallace (40). During the edematous phase of the disease the sodium concentration of the sweat was increased, but the sweat rate was reduced, resulting in an actual decrease in the total amount of sodium excreted. After diuresis, either spontaneous or induced by the administration of ACTH, the rate of sweating and sodium content of the sweat returned to normal.

The mechanism of sodium retention in congestive heart failure has received considerable attention. Hughes and his co-workers (41) studied the concentration of sodium in sweat in 14 patients with congestive heart failure. Of these, four had extremely low concentrations. Berger & Steele (42) also observed a decreased sweat sodium concentration and suggested that retention of sodium was caused by an excess of DOCA-like hormone in congestive heart failure. Robinson & Robinson (21), however, have expressed doubt over this interpretation and attributed the low sodium levels obtained to salt depletion as a result of therapy with ion exchange resins. This viewpoint was supported by Reynolds (43) who found no decrease in the concentration of sodium in the sweat in 48 patients with congestive heart failure. In fact, one group of patients in this series with both salt and water retention had sweat sodium concentrations significantly higher than the normal controls. After treatment of these patients the sweat sodium decreased in concentration. These findings have been confirmed by Haugen (44). Unfortunately, the rate of sweating was not determined in these studies. This is important in that a high concentration of sodium in the sweat does not entirely eliminate

to the secretory cells within 24 hr., to the basal cell of the duct in 48 hr., and to the luminal cell of the duct in 72 hr. SPDR granular material is replaced within the small dark cells in 72 hr. Tbe gland appears entirely normal within five days.

Daily 6 hr. episodes of profuse sweating for five consecutive days produced a remarkable series of cytologic changes. After the second day of sweating, glycogen was no longer depleted from the secretory coil. The large pale cells, however, showed further atrophy. After the third episode of sweating, marked vacuolization of the secretory cells was seen, but again no loss of glycogen. Many multinucleated large pale cells were present, presumably as the result of fusion of adjacent large pale cells. Glycogen had returned to the cells of the duct and was unaffected by sweating. After four days a definite diminution in the number of large pale cells was observed, as well as further atrophy, vacuolization, and multinucleation of those remaining. Granular material was present in the small dark cells in its presweating concentratioo. After five days, in addition to an accentuation in the changes seen in the large pale cells, marked nuclear pyknosis was present in the large pale cells, especially in those containing multinuclei.

In a more recent study (32), it became apparent that many of the changes observed in the sweat glands as a result of repeated episodes of sweating were dependent on the salt intake of the experimental subject. In one group, for example, given 30 to 40 gm. of NaCl orally per day, sweating did not produce depletion of glycogen from the secretory coil, and the cytologic changes were not as marked as was previously observed. In contrast, a group of subjects depleted of salt for one week (by means of a rice and fruit diet) prior to sweating showed loss of glycogen with each episode of sweating and more marked cytologic changes, especially involving the large pale cells.

Mainly as a result of the work of Conn (33), it has been generally accepted that acclimatization, the process by which the sweat gland develops the ability to decrease its output of sodium chloride after repeated exposures to a hot environment, is clearly the result of increased adrenal cortical activity. Conn postulated that initial exposure to heat results in excretion of concentrated sweat leading to salt depletion. This, in turn, stimulates the adrenal cortex. The increased excretion of salt-active adrenal cortical steroids, acting in some unknown way on the sweat gland, reduces the sodium excretion in the sweat. This hypothesis was supported by demonstrating that the administration of deoxycorticosterone acetate (DOCA) to unacclimatized men results in the excretion of sweat with a lowered sodium concentration on first exposure to a hot environment. Furthermore, there is a low concentration of sodium in the sweat in patients with Cushing's syndrome (34). More recently, it has been shown that salt depletion per se produces cytologic changes in the sweat gland even in the absence of sweating (32). These changes consist of atrophy of the secretory cells and absence of glycogen in the duct. Since salt depletion increases the excretion of aldosterone by the

the sweat, but these were not as high as those observed in cystic fibrosis. The mechanism of this phenomenon is entirely unknown.

Of course, the reasons for studying the human eccrine sweat gland, sweating, and sweat are multiple and range from interests in bioclimatology and environmental physiology to specific diseases of the sweat apparatus itself. Thus, perhaps it is understandable that a review of this sort would have difficulty in finding a common denominator on this subject. It is not usual in medical science for so much work to have been done on the chemistry of a secretion and on the physiology pertaining to the secreting structure itself when, at the same time, so little attention has been paid to the actual cells that produce these significant chemical and physiologic alterations in man. It is even more unusual in medical science for investigators to bypass a secretory structure so readily available for correlative studies of structure and function in man. Since it is now known that this eccrine sweat gland undergoes cytologic and histochemical changes as it sweats, a consideration of these changes becomes essential to a proper interpretation of the total picture of sweating abnormalities. An ideal observation on sweating, then, should include a correlation of function, sweat chemistry, and microanatomy after standardized stimuli, and with carefully understood or controlled internal and external environments. Perhaps much of the confusion, varied interpretation of results and seeming scientific paradoxes in this field may thus be avoided in the future.

LITERATURE CITED

1. Montagna, W., *The Structure and Function of Skin*, 117–18 (Academic Press, Inc., New York, N. Y., 1956)
2. Haimovici, H., *J. Appl. Physiol.*, 2, 512–21 (1950)
3. Lobitz, W. C., Jr., Holyoke, J. B., and Montagna, W., *J. Invest. Dermatol.*, 22, 157–58 (1954)
4. Lobitz, W. C., Jr., Holyoke, J. B., and Montagna, W., *J. Invest. Dermatol.*, 23, 329–44 (1954)
5. Lobitz, W. C., Jr., Holyoke, J. B., and Brophy, D., *Arch. Dermatol.*, 72, 229–36 (1955)
6. Charles, A., *J. Invest. Dermatol.*, 34, 81–88 (1960)
7. Lee, M. M., *Anat. Record*, 136, 97–105 (1960)
8. Dobson, R. L., *J. Invest. Dermatol.*, 35, 195–98 (1960)
9. Holyoke, J. B., and Lobitz, W. C., Jr., *J. Invest. Dermatol.*, 18, 147–66 (1952)
10. Jirka, M., and Kotas, J., *Clin. Chim. Acta*, 2, 292–96 (1957)
11. Dobson, R. L., and Abele, D. C., *The Effect of Salt Intake on Composition of Eccrine Sweat* (To be published)
12. Ackermann, A., Cited by Rothman, S., in *The Physiology and Biochemistry of the Skin* (Univ. of Chicago Press, Chicago, Ill., 1954)
13. Lobitz, W. C., Jr., and Mason, H. L., *Arch. Dermatol. and Syphilol.*, 57, 907–15 (1948)
14. Schwartz, I. L., Thaysen, J. H., and Dole, V. P., *J. Exptl. Med.*, 97, 429–37 (1953)
15. Schwartz, I. L., and Thaysen, J. H., *J. Clin. Invest.*, 35, 114–20 (1956)
16. Bulmer, M. G., and Forwell, G. D., *J. Physiol.*, 132, 115–22 (1956)
17. Thompson, N., *Clin. Sci.*, 19, 95–107 (1960)
18. Lloyd, D. P. C., *Proc. Natl. Acad. Sci., U. S.*, 45, 405–9 (1959)
19. Thaysen, J. H., and Schwartz, I. L., *J. Clin. Invest.*, 34, 1719–25 (1955)
20. Gerking, S. D., and Robinson, S., *Am. J. Physiol.*, 147, 370–78 (1946)
21. Robinson, S., and Robinson, A. H., *Physiol. Revs.*, 34, 202–20 (1954)

the possibility of sodium reteotion. For example, it bas been shown in nephrosis that altbougb the sweat sodium concentration is elevated, the rate of sweating is reduced so that there is an actual decrease in the amount of sodium excreted. A similar effect could be present in congestive heart failure. Tbis is supported by the work of Burch (45) who showed tbat the rate of sweating is reduced in coogestive beart failure. After treatment the rate of sweating returned to oormal.

Io a study of maoy patients witb hypertension, Davies & Clark (46) found a few with lowered sweat sodium conceotratioos. These patients were studied io both winter aod summer to be certaio that the low sodium concentrations observed were not merely the result of acclimatization. Of interest was the observation that all the patients with low sweat sodium concentratioos were women with central obesity, rapid weight gain, and menstrual abnormalities. This combination of signs has been termed tbe "eodocrine hyperteosive syndrome." Since the otber bypertensive patients studied had normal sweat sodium concentrations, the autbors suggested that the salt-retaining hormone of the adrenal cortex is not hyperactive in the majority of patients with hypertension but that it may be hyperactive in tbe "endocrine bypertensive syndrome."

Of all the diseases associated with changes in sweating, cystic fibrosis of the pancreas has attracted the most attention by far. Following tbe observation by Kessler & Anderson (47) that children with cystic fibrosis are especially liable to heat exhaustion, it has been sbown that there is an increased electrolyte cootent of the sweat in this disease. In a representative study (48), the concentration of sodium and chloride in the sweat of normal children was found to be in tbe range of 7 to 38 m.eq./l. In children with cystic fibrosis there was a marked increase in sweat sodium and chloride, approaching concentrations of almost 200 m.eq./l. It has also beeo shown that parents and siblings of children with cystic fibrosis may have increased concentrations of electrolytes io the sweat (49). Tbe sweating abnormality in cystic fibrosis has been recently studied in detail in ao adult (50). Even with repeated daily episodes of sweating there was no tendency toward acclimatization. Sodium concentrations in the sweat varied from 60 to 225 m.eq./l. The highest values were obtained after several hours of sweating. The excretion of potassium, urea, and lactate were normal. The structure of the sweat gland in cystic fibrosis of the pancreas has beeo incompletely iovestigated. It appears, however, that there is a marked increase in the amount of mucoprotein within tbe small dark cells of the secretory coil (50). Cbildren with chronic lung disease but without pancreatic abnormalities were also investigated (48). A moderate but significant increase in sweat sodium and chloride was fouod in many of these children.

The electrolyte content of the sweat in patients with asthma and other allergic diseases has been reported (51, 52). The majority of the patieots studied had significantly elevated conceotrations of sodium aod chloride in

MEDICAL ASPECTS OF SPACE FLIGHT

By A. H. Schwichtenberg, M.D.
Lovelace Foundation for Medical Education and Research, Albuquerque, New Mexico

One of the truly great milestones of human progress in this era is the beginning of space exploration which is now well under way. Numerous satellites placed in orbit near the earth have yielded vast amounts of new information about nearby space and about the earth itself. The knowledge of this has been obtained by means of ingenious sensing devices coupled with remarkable achievements in radio telemetry which makes possible remote measurements in the satellite that are transmitted back to earth. In addition, space probes have been directed toward the moon and some have gone into orbit about the sun. Similar radio telemetry information has been obtained on the environment of space at regular intervals.

Space exploration and its implications have become the subject of public discussion from the scientific, political, social, economic, and vital national defense points of view. Informed individuals have pointed out that a well-conceived space program, together with strong leadership and an essential national determination to excel in this costly effort, will have undreamed-of benefits to mankind.

As a group, physicians with their exceptional scientific training and broad backgrounds appreciate the magnitude of the great advances in the physical sciences, mathematics, and computers as well as those in engineering, technology, and in the life sciences which are prerequisite to these achievements. However, they have been unusually slow in adapting many of these advances to medical research, instrumentation, and medical practice. This has come about largely because of the limited communications between highly specialized physical scientists and engineers and physicians. The imminence of manned space flight as the next major step forward leads to the desirability of a review of our status in the life sciences, including medicine, necessary to support this effort. This paper will include primarily a consideration of the various human stresses involved in space flight so far as they are known and their medical implications. In addition, selection of astronauts will also be discussed.

In a paper such as this it is not possible to include other than selected general references; consequently, references have not been given throughout the paper since the number would have become unwieldy. The bibliography lists a few carefully selected general references that can serve as points of departure for further exploration of subjects of special interest. It is not possible to elaborate on the environment of space as it is known at this time; however, any successful space craft must protect the individual with reasonable safety and comfort from all elements of this remarkably hostile environment. The bulk of factual information is of comparatively recent origin and

22. Dole, V. P., Stall, B. G., and Schwartz, I. L., *Proc. Soc. Exptl. Biol. Med.*, 77, 412–15 (1951)
23. Collins, K. J., Sargent, F., and Weiner, J. S., *J. Physiol.*, 148, 592–614 (1959)
24. Sargent, F. (Personal communication)
25. Robinson, S., Gerking, S. D., Turrell, E. S., and Kincaid, R. K., *J. Appl. Physiol.*, 2, 654–62 (1950)
26. Lobitz, W. C., Jr., and Osterberg, A. E., *Arch. Dermatol. and Syphilol.*, 56, 462–67 (1947)
27. Bass, D. E., Mager, M., and Barrueto, R. B., *J. Appl. Physiol.*, 14, 431–34 (1959)
28. Yuyama, H., *Japanese J. Dermatol. Urol.*, 37, 134 (1935)
29. Shelley, W. B., and Mescon, H., *J. Invest. Dermatol.*, 18, 289–301 (1952)
30. Dobson, R. L., Formisano, V., Lobitz, W. C., Jr., and Brophy, D., *J. Invest. Dermatol.*, 31, 147–59 (1958)
31. Dobson, R. L., and Lobitz, W. C., Jr., *J. Invest. Dermatol.*, 31, 207–13 (1958)
32. Dobson, R. L., Abele, D. C., and Hale, D. H., "The Effect of Salt Intake and Repeated Episodes of Profuse Sweating on the Human Eccrine Sweat Gland," *J. Invest. Dermatol.* (In press)
33. Conn, J. W., *Advances in Internal Med.*, 3, 373–93 (1949)
34. Conn, J. W., *Arch. Internal Med.*, 83, 416–28 (1949)
35. Conn, J. W., Louis, L. H., Johnston, M. W., and Johnston, B. J., *J. Clin. Invest.*, 27, 529–30 (1948)
36. Grønbaek, P., *Acta Endocrinol.*, 18, 563–64 (1955)
37. Moseley, A. J., Fitzbugh, F. W., Jr., Hughes, D. J., and Merrill, A. J., *Am. J. Med.*, 9, 259–60 (1950)
38. Dobson, R. L., and Abele, D. C., *The Sweat Gland in Hypothyroidism* (To be published)
39. Eisenmenger, W. J., Blondheim, S. H., Bongiovanni, A. M., and Kunkel, H. G., *J. Clin. Invest.*, 29, 1491–99 (1950)
40. Warming-Larsen, A., and Wallace, W. M., *J. Clin. Invest.*, 30, 680 (1951)
41. Hughes, D. J., Turner, H. H., Moseley, A. J., and Merrill, A. J., *Am. J. Med.*, 7, 249 (1949)
42. Berger, E. Y., and Steele, J. M., *J. Clin. Invest.*, 31, 451–56 (1952)
43. Reynolds, T., *Proc. Soc. Exptl. Biol. Med.*, 79, 118–21 (1952)
44. Haugen, H. N., *Scand. J. Clin. & Lab. Invest.*, 9, 116–21 (1957)
45. Burch, G. E., *Am. J. Med. Sci.*, 211, 181–88 (1946)
46. Davies, D. F., and Clark, H., *Circulation*, 2, 494–502 (1950)
47. Kessler, W. R., and Anderson, D. H., *Pediatrics*, 8, 648–55 (1951)
48. Weeks, M. M., and Brown, G. A., *Arch. Diseases Childhood*, 33, 74–77 (1958)
49. Summer, G. K. (Personal communication)
50. Dobson, R. L., and Summer, G. K., (Unpublished data)
51. Lobitz, W. C., Jr., and Osterberg, A. E., *J. Invest. Dermatol.*, 6, 63–74 (1945)
52. Hsia, D. Y., Driscoll, S. G., Greenberg, D., Lee, T. C., and Lanoff, G., *Clin. Research*, 6, 319–20 (1958)
53. Weiner, J. S., and Hellmann, K., *Biol. Revs., Cambridge Phil. Soc.*, 35, 141–86 (1960)
54. Yoshimura, H., Ogata, K., and Itoh, S., Eds., *Essential Problems in Climatic Physiology*, 1–299 (Nankodo Publ. Co., Ltd., Kyoto, Japan, 1960)

orbits around the earth as contemplated in the Mercury project, acceleration is probably the most important of several physical stresses imposed on man. During the last war, extremely important information was accumulated on the effects of acceleration on man by the use of several human centrifuges at the Mayo Foundation, at Wright Field, the Naval Aeromedical Laboratory, the high-speed sled tests using the tracks at Holloman Air Force Base, and others. Additional information is being collected on the largest human centrifuge in the free world at the Naval Acceleration Laboratory at Johnsville, Pennsylvania.

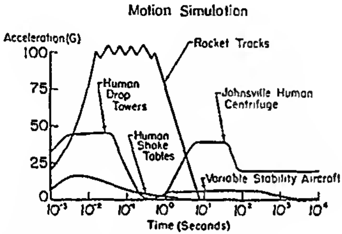


FIG. 1. Motion Simulator—Devices for Producing Acceleration. (U. S. Naval Aviation Medical Acceleration Laboratory, Johnsville, Pa., from Carl C. Clark, Ph.D.)

Acceleration is measured in terms of g which is the normal gravitational force acting upon our body and is equal to our mass or weight. If a force equivalent to 2 g should act upon an individual, he would, in respect to the direction of the force, weigh twice as much. The most important measurements in acceleration are the rate of onset in g's per second, the direction of the g forces with respect to the long axis of the body and the time at peak g. Additional factors may also be of prime importance in relation to human tolerance limits to acceleration, such as the combined effects of the other stresses which may be imposed at the same time.

The normal young adult male can tolerate about 5 g for 5 sec. before blacking out, owing to pooling of blood in dependent portions of the body. Three g's may be tolerated for about 1 hr. and Dr. Clark of Johnsville Acceleration Laboratory tolerated 2 g for 24 hr. Under this acceleration he noted headaches, anesthesia of a finger, increased water retention, increased white blood count, and reduced motivation. Head motions must be kept minimal however, because of the coriolis effect on the semicircular canals which causes disorientation and nausea. Had Clark been in a space vehicle accelerated at this level for 24 hr., he would have attained a calculated veloc-

has been obtained from very high-altitude balloon flights to the 100,000-ft. levels, from sounding rockets up to several hundreds of miles, and from various space probes, at least one of which has gone many millions of miles from earth with telemetered information available at regular intervals. Considerable information has been obtained from Russian publications concerning their space flights, some of which have been with animals as have many from this country. Space technology, astrophysics, astronautics, astrobiology and related theoretical studies constitute fascinating and dynamic subjects of unusual general interest to physicians.

Clinical Medicine Versus Aerospace Medicine

Clinical medicine concerns itself mainly with the stresses imposed on the human organism by various disease processes, pathologic states, injuries, toxic conditions, abnormal metabolic processes, psychosomatic, and psychiatric conditions. The diagnosis of these conditions and appropriate corrective therapy are most important. In aerospace medicine by contrast, the well-known techniques and standards from clinical and laboratory medical practice are employed to assist in the selection of individuals who are essentially free from the stresses of concern in clinical medicine. Instead, the specialist in aerospace medicine is preoccupied with the external stresses imposed upon the human organism by the circumstances of flight, both within the atmospheric envelope of our earth as well as in the environment of space beyond. The study of these stresses and their effects upon the human organism requires a much broader multidisciplinary approach and concept than previous medical, aeromedical, and related basic research. For this reason, a physician who is interested in this field must have additional training in aviation medicine, physiology, and psychology as well as an unusually excellent foundation and aptitude in the basic sciences, particularly mathematics, together with a willingness to work as a team member with other scientists, engineers, and technicians. This may well become a pattern for much of the future research in medicine because of the ever-increasing breadth of skills and fields of knowledge required.

The stresses most important in space flight are: acceleration, heat, vibration, radiation, decompression, hypoxia, weightlessness, noise, and illumination as well as those concerned more directly with the operation of the space craft itself, such as the atmosphere, day-night and rest-work cycles, diet, hygiene, fatigue, and psychological factors. Human tolerances and limitations must be determined to assist design engineers in the development of space vehicles having an environment in which man can carry out his functions with reasonable safety and comfort.

Acceleration

In projecting manned space vehicles into space environment either in relatively short ballistic flights lasting only a few minutes or for a few 90-min.

The engineers must design space vehicles so that the man will not be subjected to forces either too closely approaching tolerance limits shown in Figure 2 on rocket boost into orbit and re-entry into the atmosphere, or those similarly approaching tolerance limits shown in Figure 3 on impact with the earth's surface on landing. This is important because of the unknown extent of the adverse effect of other combined stresses on these tolerances. The

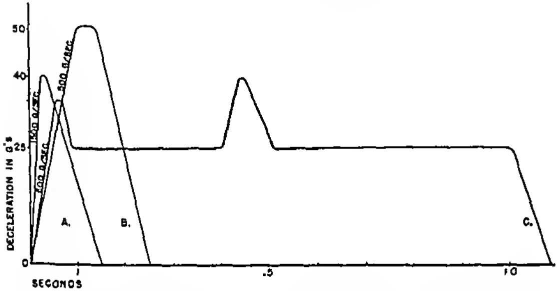


Fig. 3. Tolerance to Deceleration. Human tolerance to linear deceleration limit for reversible incapacitation to:

A. Rate of onset
B. Magnitude
C. Duration of decelerative force in the forward facing seated position

effect of extended periods of weightlessness on subsequent g tolerance is unknown.

These data have wide application in preventive medicine because they provide physicians and engineers with precise information that can be used in automotive and seat belt design. The effective application of this knowledge can save hundreds of lives and prevent many serious injuries now sustained daily by individuals involved in automobile accidents.

Heat

So far, the measured heat levels maintained within orbiting capsules appear to be within tolerable levels. Heating of space vehicles due to aerodynamic heating from air friction has not proved a very serious problem during the boost phase because speeds do not reach high values until the vehicle is largely beyond the earth's atmospheric envelope. The only way heat can be dissipated in space is, of course, by radiation. That side of the capsule facing the sun will become very hot while that facing away will be-

ity of 3.8 million miles per hour and would have traveled 45 million miles. Selected music provided an important distraction from generalized discomfort.

Individuals who have been subjects for acceleration studies have described the direction of g forces by using the direction in which the eyeballs tend to be displaced. "Eyeballs down" means upward acceleration with relation to the long body axis and is the same as positive acceleration. "Eyeballs up" is the reverse. "Eyeballs in" is foreward transverse acceleration and "eyeballs out" is backward transverse acceleration. "Eyeballs right" or "left" would similarly refer to transverse lateral acceleration.

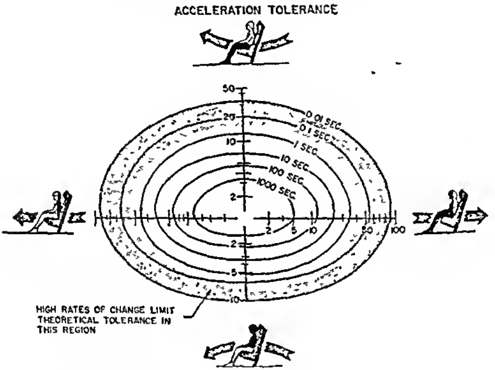


Fig. 2. Acceleration Tolerance. (After Dr. Albert E. Lombard, Jr., McDonnell Aircraft Corp.)

The study of g forces of short duration with rapid onset and high peak values acting in various directions cannot be accomplished on the human centrifuge because of power and structural limitations. For such studies, Colonel John P. Stapp and associates at the United States Air Force Acceleration Laboratory at Holloman Air Force Base, have used the high-speed rocket-propelled sled, together with an ingenious system of water brakes for the work on tolerance to impact or high g forces, applied with very rapid onset and with very short time durations. Figure 3 shows the limits of humao tolerances to such impact type of accelerative forces.

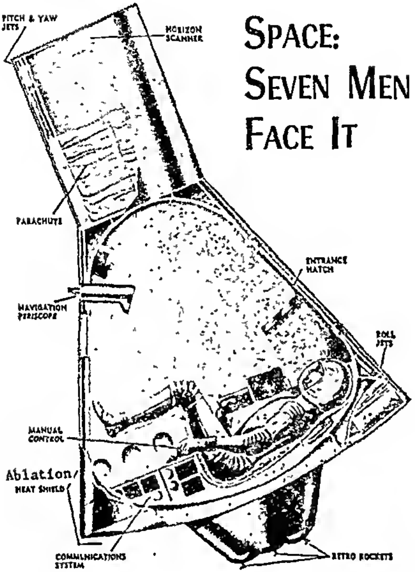


FIG. 4. Mercury Space Capsule. Sometime before mid-1961, this aerodynamically unstable, 10-foot tall (6-foot diameter) capsule, harnessed to a Redstone rocket, will carry one of America's seven astronauts briefly into space. This cut-away drawing shows the vital parts of the capsule, which will later be used atop an Atlas in a bid to establish the first manned satellite. Not streamlined like an airplane, engineers have designed faults into the capsule to *slow it down* and keep it cool *as it re-enters* the earth's lower atmosphere. *To offset its instability*, either an automatic pilot or the astronaut himself, can control (from a box near his right hand) the capsule's yaw, pitch, or roll. A special shield, insulation and air conditioning will cut the outer skin's heat from 1,200 degrees fahrenheit to interior reading of about 120 degrees.

come very cold. Unless equalized by some method or provided for by construction this will set up stresses due to expansion and contraction of the capsule structure which in turn may increase the leak rate of the air within. The problem can be resolved by either slowly rotating the capsule to heat and cool all surfaces evenly, or by a heat exchange mechanism between the hot and cold sides and also by the use of reflective paints or a combination of these. Rotation of space craft to equalize heating and provide some artificial gravity may well have undesirable side effects in orientation with head movements.

On re-entry to the earth's atmospheric envelope, heating due to air friction or aerodynamic heating becomes an extremely serious problem. This causes most meteors to flash into incandescence and vaporize. The development of successful ways to avoid this for missiles and manned space craft travelling at orbital or near orbital velocities of almost 18,000 mph or escape velocities of over 25,000 mph, is difficult. In the Mercury capsule this has been accomplished by the use of an ablation type of heat shield over the base of the capsule.

Precooling of the capsule prior to re-entry to the earth's atmosphere will be accomplished. This stress has been shown to be within human time-temperature tolerances by experiments with volunteers in space suits in heat chambers simulating the calculated times and temperatures of the capsule in its re-entry. These time-temperature calculations have been verified by actual test flights of the unmanned and monkey-containing Mercury capsule. Shown in Figure 5 are heat-time tolerances for human beings in light, one piece clothing.

Instead of the ablation shield used in the Mercury capsule, another method of keeping a capsule from burning up is the use of a heat sink composed of materials like beryllium which absorb large amounts of heat. This must be separated from the base of the capsule after re-entry. There is still an additional important method of dissipating the heat of re-entry into the atmosphere. This involves the use of newly developed materials with extremely high heat resistance coupled with the use of stubby winged glider type vehicles (Dyna-Soar), which can skip in and out of the outer atmosphere on its re-entry. This permits alternate heating and cooling by radiation while gradually slowing down for final re-entry into the earth's atmosphere. The wings will also permit some soaring capability when within the atmosphere, thus providing choice of landing sites over a radius of many hundreds of miles.

Vibration

Vibration has been a matter of concern as a human stress only recently. Most of the work has been done in military aeromedical laboratories or under their auspices in other laboratories. It is encountered especially during boost and re-entry phases of rocket flight when it is superimposed on the

laboratories. In this vacuum one finds meteors and radiation of all kinds travelling in all directions. One advantage of low-altitude orbits is that the space vehicle is shielded from almost one-half of these factors by the earth.

Meteors and meteorites are potentially dangerous to space vehicles in proportion to their relative speed, mass and, as a consequence, the energy released on impact with the skin of the space vehicle. The damage ranges

CYCLES PER SECOND	SYMPTOMS						
	Abdominal Pain	Chest Pain	Testicular Pain	Head Symptoms	Dyspnea	Anxiety	General Discomfort
1					XXXXX XXX		XXX
2					XXXXX XXX		XXXX
3	XX	XX			XXXXX	X	XXXXX
4	XX	XX		XX	XXX	XX	XXXXX
5		XXXX				X	XXXXX X
6	XXX	XXXX		X			XXXX
7	XX	XXXXX	X	X			X
8	X	XXXX		X		XX	XXX
9	XX	XXXX			X		XXXXX
10	X	X	XXX	XX		X	
15							XXXXX XXX

Fig. 6. Criteria of Tolerance to Vibration. WADC Tech. Rpt. 59-391. (From Zeigenruecker.)

from gradual etching of unprotected optical surfaces and the polished skin of the vehicle from dust particles, to small craters from slightly larger masses, to small punctures, and finally catastrophic explosions if collisions with sizeable meteors occur. Whipple developed the concept of a meteor bumper. It will vaporize most of the smaller meteorites on impact so that hull penetration probability is reduced. The calculated probability of hits on a space craft by a meteorite capable of causing a puncture has been variously estimated from once in a month or so to once per year. On one occasion, 17 impacts were recorded by telemetered sound pickups on a 50-cm. spherical

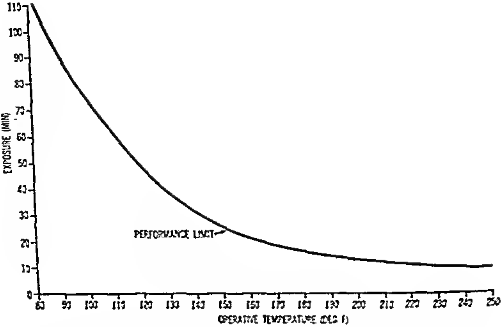


FIG. 5. Heat-Time Tolerances for Humans. For studies of human performance under heat loading, tolerance time is related to operative temperature. (E. T. Carter, Space/Aeronautics, July 1959.)

accelerative forces. Recently, studies have been made using the shake-table as a laboratory instrument. Here the subject is firmly fastened to a seat on a table which moves in a sinusoidal manner with an excursion of about one-half inch at various cycles per second (cps). These studies are still in the early stages but indicate that organs and organ systems of the body seem to have natural frequencies so that marked harmonic to-and-fro movements are set up as a result of certain vibrational frequencies. These movements place tension upon ligamentous attachments and if continued, depending on the frequency, produce cardiac pain, nausea, vomiting, and even hematuria and bloody stools have been reported. Figure 6 indicates frequencies and related symptoms. The range from 2 or 3 to 30 cycles per second appears to be the most important physiologically though there is another region around 40 cps which has adverse effects as well. In spaceship design, as well as in industry, structures and operations having these vibrational frequencies should be avoided or the occupant or worker should be shielded from their effect by suitable vibration-absorbing or dampening devices.

METEORITES

The environment of space provides a very hard vacuum of 10^{-14} mm. Hg as compared to about 10^{-11} mm. Hg as the best that can be obtained in earth

these conditions, bubbles of carbon dioxide and nitrogen come out of solution in the tissues because the pressure is diminished more rapidly than the excess nitrogen in solution can be eliminated through the lungs. Armstrong proved that just as it was known to be unsafe to reduce underwater pressures by more than one-half at a time with a suitable period for adjustment, so also

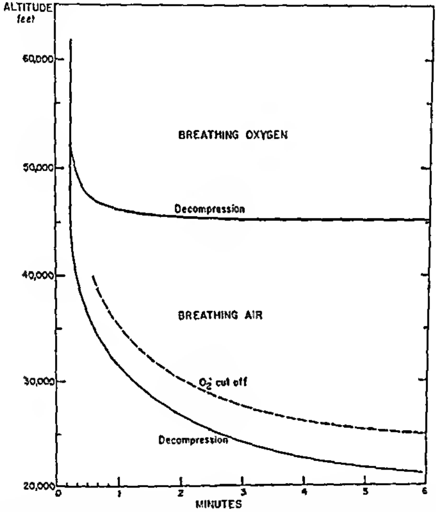


FIG. 8. Time of Useful Consciousness. Solid curves: time of useful consciousness at altitude after rapid decompression breathing air (below) and breathing oxygen (above) in pressure cabin.

Interrupted curve: time of useful consciousness after separation from oxygen supply in unpressurized cabin.

was it unsafe and symptoms were produced when the atmospheric pressure was reduced by more than one-half as in high altitude aircraft flights without adequate cabin pressurization. He called this aeroembolism.

In either case, the symptoms are similar be they in divers, pilots, or occupants of disabled space vehicles and aircraft, and depend upon where in the

space probe in 10 min. However, no knowledge is available to determine any damage resulting from these impacts. Much more information from space as well as from the laboratory is required in this area to assess properly the risks and probability of damage as well as how best to provide protection.

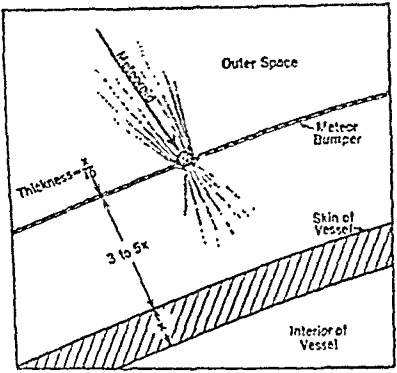


FIG. 7. Meteor Bumper by Whipple.

HYPOXIA AND DECOMPRESSION

If the space vehicle is punctured by a meteorite, leaks will occur and if not repaired immediately the passengers will be subject to hypoxia and decompression or aeroembolism. There is limited time available as illustrated by Figure 8, showing time of useful consciousness at various atmospheric pressures. For space vehicles the actual time available will depend upon the rate of pressure loss. The time during which pressure is lost is mathematically determined by the size of the hole, the volume, and air pressure of the space vehicle and the volume of stored gas which can be released per minute in an emergency. The symptoms and dangers of various levels of hypoxia are well-known to physicians and will not be elaborated upon in this paper.

Somewhat less well-known is another effect of diminished atmospheric pressure related to decompression sickness as seen in divers, caisson workers and, more recently, in skin divers who remain at considerable depth and pressures for some time and then return to the surface too rapidly. Under

belt with energies of about 500 MEV or higher, are similar to those produced by a cyclotron on earth. It requires about 1 cm. of lead per MEV to shield against them. Thus, 400 MEV protons require about 4 cm. of lead for reasonable shielding. Such a minimum shell of lead 4 m. in diameter will weigh around 5 tons. Much larger boost rockets will be necessary before such additional loads can be projected into orbit. The softer radiation more character-

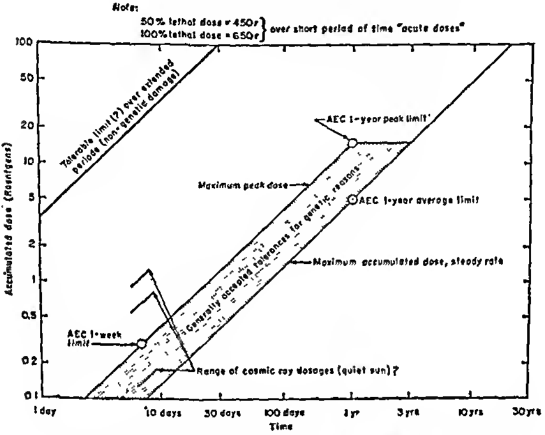


FIG. 9. Human Tolerances to Radiation. Select Congressional Committee, Staff Report, Astronautics and Space Exploration, 85th Congress, 1959.

istic of the outer Van Allen belt can be largely shielded by 1 or 2 mm. of lead though re-radiation from the hull may present some problems. An optimized radiation shield including the meteor bumper concept is shown as Figure 10.

The medical implications of this level of radiation are obvious to physicians. However, exact determinations of relative biologic effectiveness (RBE) of radiation in space are limited by insufficient detailed knowledge of the spectrum and the inability to reproduce all types of radiation in earth laboratories. In the equatorial plane at about 2000 miles altitude in the inner Van Allen belt, the exposure to man, if unshielded, is thought to be on the order of 5 to 10 or more r per hr. which would approximate the maximum allowable yearly exposure as now estimated. Until much more powerful

body tissue the bubbles form. Symptoms may be primarily neurological, respiratory, joint pains, or skin sensory disturbances and they are often combined. They may be mild and transient, or increasingly severe and occasionally produce permanent disability or death. Since nitrogen is about five times as soluble in oils and fats, individuals who are stout are usually more rapidly and seriously involved than lean individuals. The symptoms can be prevented or delayed by prebreathing 100 per cent oxygen for about 2 hr. during which time most ol the nitrogen is removed from the body. This has long been used as a protective measure by military pilots who fly at extreme altitudes.

Until recently, with the advent of commercial jet transports which operate most economically between 30,000 and 40,000 feet, the possibility of exposing other than military pilots and crewmen to decompression did not exist. Improvements in aircraft design and pressurization system reliability following earlier accidents has all but eliminated this possibility in civilian air liners. Until the extent of this problem has been determined by actual experience, occupants of space capsules during the early space flights will wear a full pressure type suit which is automatically inflated, should this become necessary. This will permit the occupant to survive even the vacuum of space for some time.

Radiation

In addition to meteoritic material which has obvious dangers to space vehicles there is a wide range of radiation both particulate as well as in the electromagnetic spectrum. Included in these are the cosmic ray particles consisting of protons and heavier atomic nuclei up to and including iron, stripped of their electrons, travelling at near the speed of light and in all directions. They originate in galactic space and from the sun, especially during solar flares. The hull of the space vehicle protects man from some, but by no means all, of this radiation. Most radiation in nearby space comes from the sun and it is now believed that the earth is in the fringe of the solar corona.

There is much more information needed on the particulate and electromagnetic radiation spectrum in space, and additional facts are learned from each satellite and space probe. At present the spectrum of radiation in space is believed to consist of electrons with energy levels ranging up to more than 1 million electron volts (MEV). There are protons with energies ranging up to 500 MEV or even higher, perhaps into the billions of electron volts, and the heavy nuclei of primary cosmic radiation have energies upwards of 500 MEV as well as beta and x-rays of 0.5 to 5 MEV of *bremsstrahlung* and nuclear origin. These originate in the hull of the space craft when molecules are struck by high-energy particulate radiation and destroyed with the liberation of x-ray and the formation of new materials. Neutrons are also present. The proton radiations, particularly in the inner Van Allen hard radiation

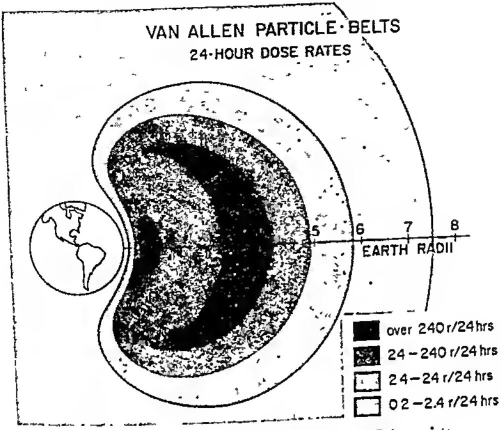


FIG. 11. Van Allen Particle Belts. Conditions: Solar quiet; Radiation as specifically measured.

utes of weightlessness, but it will not be until orbital flight is achieved that any real information will be available covering several hours' exposure. Based upon the limited experiences gained from animal and monkey shots, it is believed that this will not prove too serious for individuals such as test pilots who have become accustomed to flight and who are trained in weightlessness insofar as this can be done in aircraft. The extent and times to which man can adapt to extended weightlessness is not yet known.

NOISE

Sound waves, of course, are not propagated in the vacuum of space though the noise of equipment within the capsule and meteorite hits on the hull will be heard by the men within the atmosphere of the capsule. The medical implications of the noise levels within the capsule are the same as on earth. Noise will doubtless reach very high levels, in excess of 120 db, during the boost and re-entry phase of space flight, and may adversely affect total tolerance to accelerative stresses when added to the other stresses at these critical periods. Communications may be temporarily impaired for this and other reasons during boost and especially during re-entry.

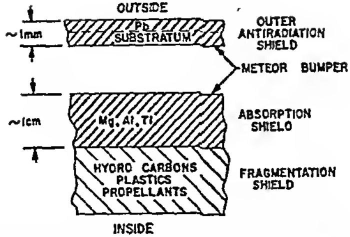


FIG. 10. Radiation Shield Design. An optimum radiation-shield design based on the presence of a large flux of low-energy protons. The outermost layer of lead reduces the radiative processes in these protons by introducing a high Coulomb barrier. This outer skin can be spaced from the inner structural skin using the meteor bumper design suggested by Whipple. The skin of the vehicle itself is made of low atomic number material to reduce the radiation from electrons; also low atomic number materials have a higher stopping power per gram per centimeter than, e.g., lead. The inner layer of hydrogenous material (fuel, propellants, plastic, water, etc.) serves to fragment heavy primaries of the cosmic radiation and thus reduce their biological effectiveness. (S. Fred Singer, U. of Maryland.)

rocket boosters are developed to project capsules with shielding into space, or possibly until some new approach to shielding is developed, earlier manned space flights must be kept to below 400 miles or over about 30,000 miles altitude, well below the inner and outside the outer Van Allen belts, respectively.

Even when space exploration is established, these high-radiation areas will probably be avoided or rapid transit times arranged to reduce total exposure. Nor can one be certain of keeping within allowable limits even in the lower radiation level zones mentioned above because major solar flares may temporarily increase radiation to dangerous levels even in these areas. Without better shielding even in the space outside the Van Allen belts, it now appears that radiation exposure may be the limiting factor on the length of trips.

WEIGHTLESSNESS

On no subject is there more speculation and less knowledge than the phenomenon of weightlessness. Experiments with aircraft flown in Kepleran trajectories can approximate weightlessness for theoretical maximums of about 75 to 90 sec. Durations of 15 to about 45 sec. are more common. Early manned ballistic flights with the Mercury capsule will produce several min-

and any system developed must be extensively tested under space conditions to prove out designs, essential stability of cultures, and long-term reliability under the conditions of space. Liquid oxygen and certain metallic superoxides and electrochemical regenerative systems are possible sources of oxygen.

Algae as food do not provide all the essential amino acids so that additional protein sources must be provided, such as perhaps fish or molluscs growing in an algeal solution, as well as perhaps a separate small hydroponics farm supporting chickens, and providing additional food variety. Human and other waste materials must either be dried and stored or incinerated in shorter flights, or re-used in the regenerative or closed ecological algae systems for longer periods. Care must be exercised to avoid placing debris in the orbital regions because of subsequent collision hazard. Periodically, a very light-weight disposable capsule may be retro-fired in such a way that the capsule together with all unwanted materials will be incinerated on re-entering the atmosphere.

The Selection of Astronauts

Knowledge and experience gained from aviation medicine was the basis for the criteria in the selection of astronauts. The Special Life Sciences Advisory Committee of the National Aeronautics and Space Administration, representatives of the United States Air Force and Naval Schools of Aviation Medicine, Aeromedical Laboratories, National Science Foundation, and civilian specialists joined in developing the selection procedures and medical criteria for astronauts. It was considered desirable for the first group to consist of specially trained experimental test pilots who met stringent physical and other requirements. They were to be less than 40 years of age and weigh less than 180 pounds, with at least 1500 hr. of pilot experience much of which was to have been in modern high-performance jet aircraft, and also possess an academic degree in engineering or the basic sciences. They were to be volunteers, very highly motivated, eager and capable of extending their knowledge and training. This was extremely important in light of the NASA concept that, in addition to becoming an astronaut, each one was to be trained to become a specialist in some phase of astronautics such as propulsion, communications, life support systems, design and construction, navigation, etc. Thus, additional previous training and demonstrated interest and aptitude in these fields were important factors as well as the individual physical and other qualifications in final selection. Later on, volunteer scientists and physicians with special aptitudes, meeting all qualifications, will doubtless be selected for space crews based upon what is learned from the early space flights and the particular mission of future space vehicles. It is believed certain that in time highly qualified women will also be included in space crews.

There were several phases to the astronaut selection process, not all of which were connected with medicine. First, records of suitable individuals

Illumination

Because of lack of material in the vacuum of space to provide reflectance, the background is very dark and the light is more intensely bright. Thus, the instruments may require intense illumination. This becomes marked above about 100 km. or 60 miles from the earth where brightness contrasts as high as 1 to 60,000 have been estimated by Campbell. This glare in sunlight and darkness in the shade may well produce eyestrain by continuous efforts of accommodation. Protective lenses will be required when viewing very bright objects in space if retinal damage and disturbances to light adaptation are to be avoided. Again, future experience alone can tell how severe this problem will be.

Cabin Environment and Atmospheres

It is clear that man cannot function satisfactorily for long periods when enclosed in a full pressure or space suit. While essential for the shorter early flights until the various hazards in space have been encountered and accurately evaluated, a "shirt sleeve" environment should be provided for longer periods. While conjectural at this time, it is anticipated that for flights of a month or so, artificial gravity need not be provided. Routine exercises with simple equipment will be required to insure physical conditioning. It has been determined that rotation of a space ship to produce artificial gravity may produce disagreeable side effects because of the disorientation caused by head movements.

It is expected that a level of compromise in cabin atmosphere will be used to provide 5 to 10 psi pressure with proper oxygen partial pressure to provide near sea-level pulmonary oxygen partial pressure levels. Pure oxygen must thus be furnished if 5 psi is used and inert gas-oxygen mixtures used for the higher pressures. While a simple pressure control is adequate for low-pressure pure oxygen systems, increasingly complex analysis and control systems are required for gas mixtures. For missions of about a month's duration, stored supplies of liquid oxygen or the use of super oxides may well prove economical weightwise. Carbon dioxide can be absorbed with lithium hydroxide, odors removed by charcoal filters and various methods can be used for toxic gas removal. Temperature and humidity will be controlled by refrigeration systems. Supercooling to freeze out carbon dioxide and toxic materials as well as remove excess moisture is a possibility. Much has been learned of toxicological problems from Naval nuclear submarine experience where prolonged exposure to a given atmosphere has shown that small amounts of substances not previously considered toxic, as used in industry, became so when breathed continuously. For longer flight periods a photosynthetic gas exchanger is desirable, using special algeal cultures or hydroponics farms to remove carbon dioxide, liberate oxygen, and provide food sources. This still requires a large and costly research and development effort

electroencephalogram, Master's double two-step, tilt table, and complete radiological studies using low-exposure techniques as well as a most complete laboratory work-up. A list of tests in addition to routine clinical tests is shown as Table I. Part of the laboratory work was to obtain base-line information for future reference.

Certain physiological tests of physical competence were added which provided specific information on the maximum physical effort the body was able to exert safely. Here a bicycle ergometer was used. This was considered a dynamic test in engineering and scores achieved were compared to those of normals by age group in terms of oxygen uptake per kilogram of total body weight and per kilogram of lean-body mass per minute at maximum effort, oxygen pulse (the number of cc. of oxygen in the cardiac stroke volume at maximum effort), circulating blood volume, and hemoglobin.

Total body water, using the tritium dilution method, total body radiation counts, made through the courtesy of the Los Alamos Scientific Laboratories, for total body potassium determinations and body specific gravities were useful in checking the lean-body mass determinations. These tests were done at the Lovelace Foundation and Clinic located in Albuquerque, New Mexico.

The stress tests, conducted at the United States Air Force Aero Medical Laboratory at Wright Patterson Air Force Base, carried the concept a step further in that here each individual was subjected to a uniform series of severe physiological stresses and the body responses carefully measured over an extended time period. These are considered roughly analogous to engineering fatigue tests. Blood pressure, pulse rate, respiration, and electrocardiograms and other measurements as appropriate were made. It now appears as though performance tests while the subject is under stress, so devised that accurate grading is possible, will in the end prove the most meaningful in selecting individuals for space crews once it is determined that they have no medically significant defects. For economy and efficiency these tests may well be combined with training and final crew selection.

The stress tests done at the Aero Medical Laboratories were intended to be meaningful in terms of the stresses to be encountered and included heat tolerance testing for 2 hr., standard acceleration tolerance, test runs on a centrifuge, altitude tolerance in a partial pressure suit at 63,000 ft. simulated in a low-pressure chamber for 1 hr., isolation tests for a brief period, modified cold pressor tests, vibration tests, performance tests in a high noise field, and additional physical competence tests. Also at the Aero Medical Laboratory, psychiatric and psychological evaluations were made as well as complete anthropological studies.

One of the noteworthy accomplishments in connection with the astronaut examinations is that all information on all the tests done was recorded on specially designed medical machine record cards of the International Business Machine Corporation (IBM) type. This provided capability of applying

meeting the criteria noted above were screened, personal interviews arranged to determine interest, and written examinations taken to indicate breadth of readily available knowledge. The physical examinations, physiologic and stress tests preceded a final selection by NASA officials at which time all factors were weighed and the first seven astronauts selected.

The medical evaluation and selection procedure of special interest to physicians made use of engineering concepts to some extent as well as long-established aeromedical and clinical medical procedures. In this sense, a most careful medical, family, and special aviation history, physical examination, laboratory, and x-ray tests were done and were considered generally in the nature of a static test of the body essentially at rest.

An unusually complete physical examination was performed on all candidates including special tests in ophthalmology, otorhinolaryngology, audiology and voice recording, nerve and neuromuscular conduction studies,

TABLE I

PHYSICAL EXAMINATION TESTS PERFORMED IN ADDITION TO USUAL ROUTING CHECKS

Exercise response	blood pressure and pulse rate
Complete ophthalmological examination	refraction, slit lamp, dark adaptation, dynamic visual acuity, fundoscopic photography
Complete ENT examination	including caloric test for equilibration function
Audiology	with tape recording of special paragraph reading and perception in 80 db white background noise
Dental examination	
Nerve	conduction times and neuromuscular conduction studies
Electroencephalographic study	
Radiology	chest, PA inspiration, PA expiration, right lateral; colon, sinuses; lumbosacral spine, stomach and oesophagus. Techniques to minimize radiation.
Laboratory	*blood grouping, hematocrit, special hematology* smear, gastric analysis, cholesterol, protein electrophoresis, catecholamines, PBI, serum electrolyte studies, 24-hour urinary steroid excretion, plus routine blood, blood chemistry and urine test.

tion in space. Many processes may well be extensively modified in surprising ways under weightless conditions and methods must be devised to overcome them. Redundant systems must be provided for safety for all vital support functions, particularly during the test phases. Nutrition and metabolism studies must be made, food requirements determined, suitable types of foods must be discovered and food preparation methods developed appropriate for the use of man and animals in space. Effective ways of overcoming loneliness and boredom, maintaining health, morale and physical fitness, and overall effectiveness under space environment, must be tested. How to provide satisfactory spatial orientation may prove troublesome. All mechanisms of human adaptation to space conditions must be studied and ways developed to facilitate those adaptive processes. Simpler and more meaningful ways of selecting astronauts and space crews must be developed on the basis of additional experience.

Radiation shielding must be tested. The final determinations of a proper work-recreation-sleep cycle and studies of day-night cycling must be verified as well as of fatigability under space conditions. Perhaps a variety of duties keeping astronauts busy during their waking hours will prove to be preferable to a recreation period. Selected music may also be helpful. Finally, the best ways of accomplishing all of the required studies in meeting human requirements in space, plus building space vehicles and boosting them into orbit within weight and dollar cost limitations, are among the most difficult problems of all.

Man-Machine Relationships

In considering the philosophy and logic of the designs of space craft from a broad medical point of view, serious consideration must be given to the role of man in his relationship to automatic or semiautomatic instrumentation sensing and control mechanisms. Automation in industry has also focused attention on man-machine relationships. Some scientists and design specialists favor unmanned space vehicles in the belief that sensing devices can be made, coupled with computers, telemetering, and actuating mechanisms which will meet the requirements. These would eliminate the necessity of carrying a man, together with the necessary environmental control and life support systems. This approach has necessarily characterized all previous satellites and space probes except those with animals and should certainly be used in future exploratory space flights where recovery is doubtful. Continuing improvements in concept and design of sensing instruments, coupled with improved computers and actuating devices and increased reliability over time, lend some support to this view.

However, most scientists are of the opinion that stable, highly intelligent trained human operators can do a number of essential things better than instruments alone and can thus add materially to mission success by monitoring automatic instruments, sensors, and computers and by making appropri-

and effort. Further developments in the art of information display in more meaningful forms, especially the use of complex relationship displays, will make the physician's task easier. This becomes increasingly important to him as the amount of medical factual data in the many specialized fields of medicine still further exceed the storage and retrieval capability of any human mind. Recording medical information, now largely unretrievable and unusable in hospital and clinic record rooms, can be revolutionized so that important advances in clinical research are made practicable. A computer of the future may well provide the practicing physicain with the equivalent of a broad consultant staff to aid him in his difficult diagnostic problems by giving more statistically probable diagnoses and perhaps even suggesting additional tests which might be done to further narrow the possibilities. This will require recording of medical information in a form compatible with computer inputs. The programming of such a computer may well become a vital additional future duty of the National Library of Medicine or the American Medical Association for which a staff or groups of outstanding medical specialists of all categories will be required to weigh and validate factual medical information. It is altogether possible that some present concepts in medicine may not stand rigorous scientific testing for validity.

ate adjustments, maintenance, and repairs which cannot otherwise be done. The technical problems of achieving unattended long-term reliability of a large number of components are staggering. Man also has inherent drives to explore new realms which are not long satisfied by mere instrumented probes. It is generally considered that the best success will be attained by optimum combinations of man and machine, making use of the better features of each, and much effort has been devoted toward achieving this end.

In comparing man with machines in a broad sense it is apparent that he excels in being able to detect very small amounts of visual and acoustic energy over quite a wide range. He is able to perceive and recognize various patterns in light and sound. He is flexible in response and, of utmost importance, is able to improvise ingenious solutions to all kinds of new and unforeseen operational situations. He can store large amounts of information over long-time periods and recall factual data at appropriate times. He has great breadth in data processing, is good at self-programming and is resistant to jamming. He can exercise judgement and decision with great flexibility and successfully apply inductive reasoning often with minimal information. He can repair and adjust any machine man can devise, and he can intelligently use outputs from many other individuals and machines that can be communicated to him. Man also has certain liabilities in that he has limited force capabilities, he is susceptible to perceptual errors through bias, and is subject to self-induced stresses, fatigue, boredom, and inattention.

Machines, on the other hand, can be designed to have far shorter response times and respond accurately and consistently to given control signals. They can be made to control and apply far larger forces smoothly and precisely over longer time periods without fatigue. They can be provided with sensors which detect phenomena outside human ranges of capability including ionizing radiation, x-rays, cosmic radiation, radio and microwaves, as well as sounds in the sub- and ultrasonic regions. Information can be stored briefly and responded to, then erased completely. They can perform many routine tasks repetitively, accurately, and consistently without fatigue or boredom. Computers can be made to do certain deductive reasoning and perform extremely rapid and complex computations as programmed and can carry on a number of operations simultaneously in extremely brief time periods. They can remain alert for long periods without wavering attention to certain signals. There are also many liabilities, the main one of which is lack of long-term reliability without routine maintenance.

The appropriate application of developments in instrumentation to medicine will bring about far-reaching changes enabling the physician to spend more time with patients. Many routine tasks of diagnostic importance can be accomplished by use of improved sensing and display devices. These instruments will be adapted from those developed for space technology. They may be used as inputs for computers that have been programmed to provide the physician with information now laboriously obtained at great expense of time

NEURAL CONTROL OF ENDOCRINE FUNCTION[1,2]

GORDON FARRELL, M.D.[3] AND ELIZABETH W. RAUSCHKOLB, M.D.
Departments of Physiology and Dermatology, Western Reserve University and University Hospitals, Cleveland, Ohio

A classification of neuroendocrine mechanisms based on the number of endocrine organs interposed between the central nervous system and the ultimate target tissue has been suggested by Rothballer (1). The classification is unique, logical, and convenient. The following summary of certain recent advances in the physiology of neuroendocrine relationships will utilize the framework of this classification, with minor modifications.

One of the important new developments is the emergence of the concept of inhibition as an endocrine function. We shall touch upon neurohumoral inhibitory mechanisms in an effort to bring these within the structure of the classification.[4]

No effort will be made to review the literature exhaustively on any one topic (i.e., adenohypophysis and adrenal cortex, or control of TSH release or of gonadotropin secretion) since several excellent recent reviews devoted to these subjects are available. The authors have selected a rather small number of publications in an effort to illustrate the general thesis of the categorization. Emphasis will be placed on unanswered questions.

FIRST-ORDER NEUROENDOCRINE MECHANISM

In this simplest neuroendocrine relationship, a neuron whose cell body is within the central nervous system secretes a substance which is carried in the blood stream to act directly on target somatic cells. The secretion of antidiuretic hormone is an excellent example. This hormone (ADH) is elaborated within the cells of the supraoptic and paraventricular nuclei, transported (possibly with continued synthesis) along the axons of these cells to the neurohypophysis and released into the blood (2 to 5). The pituicyte has an

[1] The survey of the literature pertaining to this review was concluded in October, 1960.

[2] The following abbreviations will be used: ADH (antidiuretic hormone); CRF (corticotropin-releasing factor); FSH (follicle-stimulating hormone); ICSH (interstitial cell-stimulating hormone); MSH (melanocyte-stimulating hormone); TSH (thyroid-stimulating hormone).

[3] Established Investigator, The American Heart Association.

[4] One might well ask, what is the *raison d'être* of inhibition in the endocrine system? Stimulation is the only necessary hormonal action if the activity of the target tissue spontaneously subsides in the absence of continued stimulation and if the rate of decline of activity (when stimulation is removed) is rapid enough to permit adequate adaptive responses. The latter may be an important limitation, and, in certain instances, prompt reversal of the effects of a stimulatory hormone by an inhibitory factor which acts on the same target tissue may be necessary for homeostasis.

LITERATURE CITED

1. *Aerospace Medicine* (The Journal of Aviation Medicine, recently renamed Aerospace Medicine, is considered to be one of the best general sources in this field and is published monthly)
2. Armstrong, H. G., Ed., *Principles and Practices of Aviation Medicine*, 4th ed. (William & Wilkins Co., Baltimore, Md., 1960)
3. Benson, O. O., Jr., Ed., *Lectures in Aerospace Medicine* (Conducted at the School of Aviation Medicine, USAF Aerospace Medicine Center (ATC), January 11–15, 1960, Brooks AFB, Texas. Available through the School of Aviation Medicine)
4. Benson, O. O., Jr., Strughold, H., Eds., "Physics and Medicine of the Atmosphere and Space," *Proc. Intern. Symposium on the Physics and Medicine of the Atmosphere and Space, 2nd Symposium*, Nov. 1958, Brooks AFB, Texas (John Wiley & Sons, Inc., New York, 1960)
5. Goodyear Aircraft Corporation, Akron, Ohio, *Air/Space Crew Environmental Data Book, GER-9523* (November, 1959)
6. Konecci, E. B., "Manned Space Cabin Systems," in *Advances in Space Science* (Academic Press, Inc., New York, N. Y., 1959)
7. McCormack, J. W., Chairman, Select Committee on Astronautics and Space Exploration, *Space Handbook: Astronautics and its Applications* (Staff Report of the Committee, Government Printing Office, Washington, D. C., 1959)
8. Proceedings, *Manned Space Stations Symposium*, (Inst. Aeronautical Sciences, April 20–22, 1960, Los Angeles, California)
9. Schwichtenberg, A. H., Flickinger, D. D., Lovelace, W. R., II, "Development and Use of Medical Machine Record Cards in Astronaut Selection," *U. S. Armed Forces Med. J.*, 10, No. 11, 1324–51 (November, 1959)
10. Slater, L. E., Ed., *The Pilot Clinic on the Instrument Requirements for Human Comfort and Survival in Space Flight Proceedings* (Foundation for Instrumentation Education and Research (FIER), October 26–27, 1959, Columbus, Ohio; FIER, 335 East 45th Street, New York 17, N. Y., 1960)
11. Summary Session of *The Astronautics Symposium* (USAF Office of Scientific Research and Convair Division of General Dynamics Corporation, February 18–20, 1957, San Diego, California)
12. White, C. S., Benson, O. O., Jr., Eds., *Physics and Medicine of the Upper Atmosphere, Proceedings* (Symposium sponsored by The Air University, School of Aviation Medicine, November 6–9, 1951, Randolph Field, Texas; University of New Mexico Press, 1952)

Although a problem arises in deciding which is stimulatory and which inhibitory in terms of effects on the melanocyte, it is convenient to think of MSH secretion as a second-order stimulatory neuroendocrine mechanism and melatonin secretion as a second-order inhibitory neuroendocrine mechanism. The end result is a nicely adjusted, facile system for the control of pigmentation which is important for lower forms of life. The MSH-melatonin relationship raises the possibility of other instances of antagonism between pituitary and pineal hormones.

Second-order neuroendocrine relationships may exist in connection with two adenohypophyseal factors; growth hormone and prolactin (1). Both hormones act directly on target cells without the necessary interposition of other endocrine tissue. However, inclusion of these two tropic hormones in this category obviously involves certain assumptions. The regulation of the secretion of growth hormone is obscure. Lesions of the hypothalamus increase the hypoglycemic response to insulin, possibly as a result of reduced growth hormone secretion (24), suggesting a stimulatory role for the hypothalamus. However, rats with transplanted pituitaries grow almost as well as do normal animals (25), and tadpoles with pituitary transplants grow faster than do controls (26). There is a suggestion from the latter findings that the hypothalamus inhibits growth hormone secretion. The concept is not unique. The hypothalamus tonically inhibits prolactin secretion according to some workers (27). Indeed, lesions of the medial basal tuberal region lead to initiation of milk secretion, as though an inhibitory mechanism were interrupted (28). On the other hand, oxytocin stimulates prolactin release, leading other workers to postulate that it is the neurohumoral factor that controls prolactin secretion (29, 30). There seems to be disagreement on this point (31). The possibility remains that both stimulatory and inhibitory neurohumors are concerned in the regulation of growth hormone and prolactin, a compromise point of view which may also have to be taken in the instance of certain other pituitary hormones, notably ACTH (*vide infra*).

Third-Order Neuroendocrine Mechanisms

The central nervous system stimulates endocrine tissue to elaborate a hormone which, in turn, stimulates a second endocrine tissue to secrete its hormones to act on the somatic target tissue. The secretions of adrenocortical steroids, sex hormones, and thyroid hormone(s) are under the control of such complex systems, which are third-order neuroendocrine mechanisms according to this classification. The possible teleological basis for such complexity has been discussed by Rothballer in terms of timing and duration of hormone action, amount of the final hormonal products released into the circulation, and increased possibilities for sensitive control (1).

Much of the credit for the original research on the neural control of such systems belongs, of course, to Harris (32, 33). The neural regulation of ACTH secretion in particular has been studied in some detail. There is no question

ill-defined role in the process (6). The hormone acts directly on renal or bladder tissue (7).

Oxytocine secretion probably belongs in the same category as that of ADH. It is secreted by neurons whose cell bodies lie within the hypothalamus and released in the neurohypophsis just as is ADH (8). Overlapping biological activity and the occurrence of transitional forms of the two hormones in some species reflect a common evolutionary pathway (9).

Second-Order Neuroendocrine Relationships

The utilization of an endocrine tissue as an intermediate step between the central nervous system and somatic cells is considered to represent a second-order neuroendocrine mechanism. A difficult decision must be attempted in the classification. How does one differentiate between endocrine tissue on the one hand, and neural tissue on the other? Rothballer takes the position that the secretion of medullary catechol amines represents a transitional stage between first- and second-order mechanisms, since the medullary cells are of neural origin. However, for purposes of simplicity, we should like to suggest that a tissue of neural origin which has lost axons and dendrites and does not subserve conduction (but rather secretes a hormone) be classified as endocrine tissue despite its embryological history. In this light, the adrenal medulla would be considered as part of a second-order neuroendocrine mechanism.

The physiology of the melanocyte-stimulating hormone has been the topic of considerable interest in the past few years. The hormone is believed to be secreted by cells in the pars intermedia in response to stimuli (neural or neurohumoral) traversing the pituitary stalk and to act directly on the target tissue, the melanocyte (10 to 14). The system can thus be classified as a second-order neuroendocrine mechanism. The hormone induces pigment granule dispersion and ultimately melanogenesis (12). It is curious that ACTH is also active in pigment dispersion and possibly in melanogenesis (11, 15, 16) although MSH has no ACTH activity. A possible explanation is found in the recent exposition of MSH structure (17, 18, 19). The sequence of amino acids in MSH is found in ACTH; MSH may be released during the metabolic degradation of ACTH. Prolactin also promotes melanogenesis and potentiates MSH action (20). Does its structure also contain the amino acid sequence of MSH?

Important developments have occurred in the elucidation of another second-order neuroendocrine mechanism, the secretion of melatonin by the pineal. Melatonin [N-acetyl-5-methoxytryptamine (21)] reverses the pigment-dispersing action of MSH by direct action on the melanocyte (14, 22, 23). Additional information is needed as to which pineal cells secrete the hormone and the manner in which these cells are stimulated. It appears that release of melatonin is governed by a neural mechanism responding to light (23).

Exploration of this concept may be very profitable, perhaps leading to an explanation of some of the discrepancies found in the literature dealing with the hypothalamic-pituitary mechanisms.

It should be pointed out that possibilities for hormonal inhibition occur at three places in a third-order neuroendocrine mechanism. In the ACTH system these would be: (*a*) within the brainstem at the cells which secrete CRF; (*b*) at the adenohypophysis to block the action of CRF or to directly inhibit ACTH release; or (*c*) at the adrenal. The feedback mechanism by which cortical steroids inhibit steroidogenesis apparently acts at all three levels (68, 69). A curious but apparently physiologically significant inhibition of steroidogenesis also results from estrogen administration (70). ACTH itself may reduce ACTH secretion (71). These latter phenomena may belong in the category of fine adjustments in the system.

The literature contains other intriguing observations whose implications can only be surmised. Why does testosterone administration increase adrenal weight in the hypophysectomized rat (72)? Do vascular changes in the hypophyseal portal system play a role in the hypothalamic-pituitary relationship (73)? Why does the pattern of certain urinary steroids (pregnanctriol, dehydroepiandrosterone) change spontaneously and independently of the others (74)? And, finally, why do certain brain lesions (particularly in the septal region) change the pattern of cortical steroids in the adrenal effluent (75)?

The systems for the control of sex hormone secretion are also third-order neuroendocrine mechanisms. As is the case with the ACTH-adrenal cortex axis, the central nervous system undoubtedly plays a major role in controlling gonadal activity. Luteinizing hormone is released at a particular time of day (independently of ACTH output) (76). There is diurnal variation in sensitivity to chorionic gonadotropins, presumably reflecting some change in central nervous system function (77). The normal estrus cycle is interrupted by reserpine (78) and the drug leads to menstrual irregularities in monkeys (79). Ovulation occurs in response to vaginal stimulation and the phenomenon is facilitated by estrogen (80). The list of such observations which implicate the brainstem in sexual activity is long, and only a few of the more recent observations are listed here.

Lesions in the ventromedian area of the preoptic hypothalamus block progesterone-induced ovulation in the chicken (81) and posterior median eminence destruction leads to testicular and prostatic atrophy and reduction of pituitary ICSH and FSH (82, 83). Lesions of the median eminence and pituitary stalk also reduce release of gonadotropins in the ewe (84). Basal tuberal lesions block copulation-induced ovulation and damage to the mammillary bodies results in refusal to mate (85). Lesions at the mesencephalic-diencephalic junction also block ovulation (86). However, the anovulatory state achieved in the chicken by lesions of the median diencephalon is temporary; a longer period of anovulation follows lesions of the preoptic hypothalamus, supraoptico-hypophyseal area or dorsocaudal thalamus, but recovery is

but that the central nervous system plays an important role in the acute responses of the adrenal cortex to stressful conditions, and in inducing cyclic changes in steroidogenesis (34, 35, 36). Transection of the brain stem profoundly alters the responsiveness of the system (37, 38, 39). The median eminence seems to be the final common pathway, perhaps the source of the neurohumoral factor for ACTH release (corticotropin-releasing factor, CRF). Lesions in the median eminence, or stalk section, prevent the acute response to stress (40 to 44), and stimulation of the ventral hypothalamus activates ACTH release (45, 46).

The corticotropin-releasing factor has not been identified as yet. Vasopressin stimulates ACTH release in intact animals and in animals bearing median eminence lesions, and it breaks through steroid blockade (47 to 51). It also acts to increase ACTH output when introduced into the third ventricle or when applied topically on grafted pituitaries (52, 53). But antidiuretic hormone has an ubiquitous action: it induces ascorbic acid depletion in decapitate rats, potentiates the action of ACTH, and has a direct ACTH-like action of its own (54 to 57). Corticosteroid levels are not necessarily increased in antidiuresis (58). Further, three groups of workers have evidence of CRF factors other than ADH from median eminence tissue or hypophyseal portal blood (59, 60, 61). The final resolution of the problem is complicated by the phenomenon of overlapping biological activity of hormones of related structure. It might be thought possible to resolve the question by purifying the various candidate factors and determining which is the most potent. Unfortunately, there is no guarantee that the most potent is the natural secretory product. Characterization of CRF as obtained from hypophyseal portal blood (61) would seem to offer the best approach to the problem.

Even should the nature of CRF be determined, perplexing questions in the regulation of ACTH release still exist. Adrenal hypertrophy occurs in response to chronic stress in rats with median eminence lesions. Arguments have been presented for two systems for the stimulation of ACTH, perhaps for the existence of two corticotropins (44, 62, 63). The isolated pituitary *in situ* (the brain removed rostral to the midbrain) has a high resting level of ACTH output, but responds to stress, thus implicating a stimulatory factor from the hindbrain, the output of which is normally restrained by higher centers (64). Stimulation of the area preoptica or upper posterior diencephalon leads to prolonged depression of cortical steroid output (46, 65). Inhibition of ACTH release may be involved, although the secretion of a factor which inhibits steroidogenesis at the adrenal level cannot be excluded. The observation that certain fractions from pineal extracts reduce steroidogenesis, probably by direct action on the adrenal (66), lends credence to the latter possibility. There is additional evidence that the pineal may be the source of factors which antagonize the adenohypophyseal hormones (67). Is it possible that a balance of stimulatory-inhibitory neuroendocrine mechanisms exists for the control of steroidogenesis just as it does for pigmentation?

inhibited at the level of the gland itself? Very little evidence exists for such a system, but there are a few suggestions in favor of the concept. A factor from serum has been described as an inhibitor of thyroid function, and purified extracts of plasma contain more TSH activity than can be accounted for in the whole plasma (108, 109). It is odd that triiodothyronine depresses thyroid function more effectively if the pituitary is present than if it is absent (110). Does the pituitary secrete a thyroid inhibitor?

The secretion of aldosterone is subject to influence by a complex system which is not as yet completely understood. There seems to be little question but that the third-order hypothalamic-adenohypophyseal pathway for the regulation of the secretion of the other corticoids participates in aldosterone control.[5] Hypophysectomy results in a quantitatively variable but qualitatively consistent drop in aldosterone output (111, 112). Ventral hypothalamic lesions which reduce cortisol secretion (presumably as a result of decreased ACTH) also lead to a reduction in aldosterone output (43, 113, 114). However, numerous instances of the independence of aldosterone secretion rates from those of the other corticoids have been documented [see reviews (115, 116)], and evidence has been presented for a circulating hormone specific for aldosterone secretion (117, 118). What remains to be determined is the chemical nature and source of the hormone that modulates the secretory activity of the adrenal in such a way as to promote aldosterone synthesis. Three groups of workers have obtained fractions from urine which selectively increase aldosterone output. These appear to be protein or lipoprotein (119, 120, 121). A lipid factor (adrenoglomerulotropin) from pineal tissue selectively stimulates aldosterone output in the decerebrate dog (122, 123). The material has been partially purified, and appears to be quite potent (124). Adrenoglomerulotropic activity appears in the vein of Galen under conditions of hemorrhage (124). The latter observations would appear to invoke the pineal as the organ responsible for the selective changes in the steroidogenesis of aldosterone. The system would be classified as a third-order mechanism. However, pinealectomy does not permanently reduce aldosterone secretion or prevent the expected response to sodium deprivation (125). As a matter of fact, removal of the pineal seems to exaggerate the effects of sodium deprivation, resulting in a near-doubling of both cortisol and aldosterone secretion as compared with controls subjected to the same conditions. A possible explanation is the existence of a pineal factor which inhibits steroidogenesis (125). It is interesting, in this connection, that while certain midbrain lesions in the cat depress aldosterone secretion, other larger lesions in the same general area result in a significant elevation in output of the steroid. These results were in-

[5] No effort has been made in this review to consider the receptors and afferent pathways subserving the control of endocrine activity. However, the extensive and well-controlled studies of Gann and Bartter and their collaborators on these aspects of the regulation of aldosterone secretion deserve comment. Their results indicate that pressoreceptors in the carotid arteries reflexly affect aldosterone output. Evidence is also presented for a cerebral chemoreceptor mechanism (143, 144, 145).

again observed (87). On the other hand, certain lesions of the anterior hypothalamus below the massa intermedia and of the amygdala induce precocious sexual activity (88, 89). A more precise localization of the area concerned in this phenomenon has been reported, placing it in the arcuate nucleus of the posterior tuberal region (90). Non-specific effects of cranial surgery may present a problem in interpretation; precocious activation of ovarian function has also been reported following sham operations (91). Taken as a whole, there seems to be a clear suggestion that both excitatory and inhibitory components exist at the first step in this complex neuroendocrine system. The locus of action of inhibition remains unknown. No one as yet has taken a firm position on the concept that a neuroendocrine inhibitor for ICSH or FSH is carried to the adenohypophysis by the portal circulation. Nevertheless, it seems a possibility worthy of exploration, particularly in view of the evidence for central nervous system inhibition of prolactin release (27). Is it possible that the precocious sexual development observed as a result of certain brain lesions is the consequence of disruption of the habenular-pineal complex, believed to be the source of an inhibitor or inhibitors of gonadal activity (67)?

A negative feedback system has been described in which the further release of gonadotropins is inhibited by the discharge of gonadotropin (or estrogen) following coitus. The locus of inhibition is probably within the brainstem as judged by concomitant EEG and behavioral changes (92, 93, 94).

The nature of the presumed neurohumor which stimulates gonadotropin release is not known. It could probably have been predicted that the posterior pituitary hormones would be invoked again, and they have been (95), but with no more assurance than in the case of ACTH or prolactin release.

The control of thyroxine secretion falls into the pattern of a third-order neuroendocrine mechanism. Destruction of an area in the anterior hypothalamus leads to diminished TSH release (96, 97, 98, 99). A neurohumor of unknown chemical nature may be secreted by cells in the area and acts on the adenohypophysis via the portal circulation, although the secretion of TSH may be less dependent on the hypothalamus than is the case with the other tropic hormones (33, 100). Once again, the suggestion has been made that the posterior pituitary hormones are involved (101), but there is little support for the concept since thyroid function is not altered by repeated administration of vasopressin or oxytocin (102). We may probably expect the same kinds of problems to arise in the study of this neurohumor as are currently being encountered in the study of CRF. Overlapping biological activity confounded by the mechanical difficulties in obtaining the neurohumor as secreted will undoubtedly delay the eventual resolution of the question.

A negative feedback system operates in the TSH-thyroid relationship as it does in the other third-order mechanisms. Thyroid hormones inhibit TSH release at both the hypothalamic and pituitary levels (103 to 107). The question arises: is there a humoral mechanism by which thyroid activity is

filtration rate. Although Smith spoke in terms of a factor that causes sodium retention (quite the opposite of what was observed in Wise & Ganong's work), the concept that the brainstem participates directly in controlling renal function is clearly supported. Is it possible that cerebral sodium wasting (136) is attributable to hypersecretion of a natriuretic factor?

A role for the midbrain in water metabolism was postulated by Gilbert, who found that lesions of the subcommissural organ resulted in reduced water intake (139, 140). The conclusions were questioned by certain workers who maintained that the effects were caused by postural changes. However, we have been able to confirm the observation in dogs who showed no postural changes whatsoever (141). Further, it has been reported that pinealectomized animals excrete a water load very slowly; the function is restored by a pineal extract indicating the presence of a diuretic factor in the pineal (142). Much additional work is needed before a final statement can be made. However, it may well be that humoral factors arising in the dorsal brainstem or its neural appendages act to affect water metabolism, perhaps as a first-order neuroendocrine mechanism.

Only time and additional research will supply the answers, but the unfolding of these newer (but perhaps paleontologically older) aspects of neuroendocrine physiology should provide fascinating reading in the years ahead.

LITERATURE CITED

1. Rothballer, A. B., *Excerpta Med., Sect. III.*, 11, iii (1957)
2. Leveque, T. F., and Scharrer, E., *Endocrinology*, 52, 436 (1953)
3. Scharrer, E., and Scharrer, B., *Handb. Anat. des Menschen*, 6/5, 953 (Springer-Verlag, Berlin, Ger., 1954)
4. Gerschenfeld, H. M., Tramezzani, J. H., and De Robertis, E., *Endocrinology*, 66, 741 (1960)
5. Weinstein, H., Berne, R. M., and Sachs, H., *Endocrinology*, 66, 712 (1960)
6. Leveque, T. F., and Small, M., *Endocrinology*, 65, 909 (1959)
7. Bentley, P. V., *J. Endocrinol.*, 17, 201 (1958)
8. Van Dyke, H. B., Adamsons, K., Jr., and Engel, S. L., *The Neurophypophysis, Proc. Symposium Colston Research Soc., 8th Pnmp.*, 65 (Heller, H., Ed., Butterworths Sci. Publs., London, Engl., 1957)
9. Munsick, R. A., Sawyer, W. H., and Van Dyke, H. B., *Endocrinology*, 66, 860 (1960)
10. Dalton, H. C., *Pigment Cell Growth, Proc., Conf. on the Biology of Normal and Atypical Pigment Cell Growth*, 3rd Conf., 17 (Academic Press, Inc., New York, N.Y., 1953)
11. Chavin, W., *Pigment Cell Biology*, 63 (Gordon, M., Ed., Academic Press, Inc., New York, N. Y., 1959)
12. Lee, T. H., and Lerner, A. B., *Pigment Cell Biology*, 435 (Gordon, M., Ed., Academic Press, Inc., New York, N. Y., 1959)
13. Lerner, A. B., and Takahashi, Y., *Recent Progr. in Hormone Research*, 12, 303 (1956)
14. Wright, R. M., and Lerner, A. B., *Endocrinology*, 66, 599 (1960)
15. Shepherd, R. G., Howard, K. S., Bell, P. H., Cacciola, A. R., Child, R. B., Davies, M. C., English, J. P., Finn, B. M., Meisenhelder, J. H., Moyer, A. W., and van der Scheer, J., *J. Am. Chem. Soc.*, 78, 5051 (1956)
16. Bell, P. H., *J. Am. Chem. Soc.*, 76, 5565 (1954)
17. Harris, J. I., and Lerner, A. B., *Nature*, 179, 1346 (1957)
18. Harris, J. I., *Biochem. J.*, 71, 451 (1959)
19. Lerner, A. B., *Nature*, 184, 674 (1959)
20. Kosto, B., Pickford, G. E., and Foster, M., *Endocrinology*, 65, 869 (1959)
21. Lerner, A. B., Case, J. D., and Heinzelman, R. V., *J. Am. Chem. Soc.*, 81, 6084 (1959)

terpreted as pointing to an inhibitory area in the midbrain or posterior diencephalon (43, 115).

It is to be emphasized that the problem is by no means solved. Efforts of other workers to obtain definitive answers have yielded little additional information. Denton and his co-workers are led to the conclusion that some structure in the mesencephalic-diencephalic area is concerned with aldosterone regulation (126). Although the work tends to support, in a general way, the concept of central nervous system control of aldosterone it contains many contradictions, and final conclusions are scarcely possible (127, 128). The dependence on the rather improbable assumption that salivary sodium/potassium ratios oecessarily reflect endogenous aldosterone secretion rates may explain some of the discrepancies in the results of these workers. The physiology of the salivary glands, particularly with regard to electrolyte handling, is sufficiently complex to preclude such a simple, albeit attractive, method of assay for endogenous aldosterone (129, 130). Davis and his collaborators have proposed the kidney as the site of origin of a factor for aldosterone secretion (131). However, the paucity of published data at this time makes impossible a final statement as to the validity of the concept. The finding of biological activity in extracts of kidney must, in general, be tempered with caution, because of the proclivity of this organ to take up and store hormones (insulin, ACTH, ADH) obviously originating elsewhere (132, 133, 134).

Clouds on the Horizon

Throughout this short review the authors bave tried to emphasize what appear to be tbe newer trends in endocrine physiology, occasionslly straying far from the path of orthodoxy. This was done with the full realization that many times what appears to be a new physiological system may eventually prove to have been only another aspect of the operation of systems already known and well documented. Nevertheless, it seems wise to be alert to the possible emergence of hitherto unkoown homeostatic mechanisms. In the literature are a few hints that this may be happening, particularly in the area of water and electrolyte balance. It was with considerable scepticism that many physiologists greeted Homer Smith's hypothesis of a brainstem hormone for the regulation of renal sodium handling (135), although Peters and his collaborators bad raised the possibility beforehand (136). It must be admitted that neither group had very much direct experimental evidence on which to base such an bypothesis. Quite recently, however, it has been reported that stimulation of an area in the ponto-medullary junction induces an immediate increase in sodium excretion not dependent on renal nerves (137, 138). The effect is presumably bumoral; if so, this probably means that the brainstem (or some structure influenced by stimulated fibers in the area) does indeed release a hormone that affects sodium reabsorption at the kidney level. The effects were much too prompt to be explained on the basis of a change in adrenal steroid output, and occurred in the absence of a change in

68. McCann, S. M., Fruit, H., and Fulford, B. D., *Endocrinology*, 63, 29 (1958)
69. Peron, F. G., Moncloa, F., and Dorfman, R. I., *Endocrinology*, 67, 379 (1960)
70. McKearns, K. W., Coulomb, B., Kaleita, E., and De Renzo, E. C., *Endocrinology*, 63, 709 (1958)
71. Kitay, J. I., Holub, D. A., and Jailer, J. W., *Endocrinology*, 64, 475 (1959)
72. Lanman, J. T., and Dinerstein, J., *Endocrinology*, 64, 494 (1959)
73. Worthington, W. C., Jr., *Endocrinology*, 66, 19 (1960)
74. Fotherby, K., and Strong, J. A., *J. Endocrinol.*, 19, 389 (1960)
75. Endröcsi, E., and Lissák, K., *Acta Physiol. Acad. Sci. Hung.*, 17, 39 (1960)
76. Schwartz, N. B., and Boswell, L. S., *Endocrinology*, 63, 319 (1958)
77. Lamond, D. R., and Braden, A. W. H., *Endocrinology*, 64, 921 (1959)
78. Barraclough, C. A., and Sawyer, C. H., *Endocrinology*, 64, 563 (1959)
79. Erikson, L. B., Reynolds, S. R. M., and De Feo, V. J., *Endocrinology*, 66, 824 (1960)
80. Sawyer, C. H., and Markee, J. E., *Endocrinology*, 65, 614 (1959)
81. Ralph, C. L., and Fraps, R. M., *Endocrinology*, 65, 819 (1959)
82. Davidson, J. M., and Ganong, W. F., *Endocrinology*, 66, 480 (1960)
83. Davidson, J. M., Contopoulos, A. N., and Ganong, W. F., *Endocrinology*, 66, 735 (1960)
84. Clegg, M. T., and Ganong, W. F., *Endocrinology*, 67, 179 (1960)
85. Sawyer, C. H., *Anat. Record*, 124, 358 (1956)
86. Critchlow, V., *Endocrinology*, 63, 596 (1958)
87. Ralph, C. L., and Fraps, R. M., *Am. J. Physiol.*, 197, 1279 (1959)
88. Elwers, M., and Critchlow, V., *Am. J. Physiol.*, 198, 381 (1960)
89. Donovan, B. T., and van der Werff ten Bosch, J. J., *J. Physiol.*, 147, 78 (1959)
90. Gellert, R. J., and Ganong, W. F., *Acta Endocrinol.*, 33, 569 (1960)
91. Herbert, J., and Zuckerman, S., *J. Endocrinol.*, 17, 433 (1958)
92. Sawyer, C. H., and Kawakami, M., *Endocrinology*, 65, 622 (1959)
93. Kawakami, M., and Sawyer, C. H., *Endocrinology*, 65, 631 (1959)
94. Kawakami, M., and Sawyer, C. H., *Endocrinology*, 65, 652 (1959)
95. Martini, L., Mira, L., Pecile, A., and Saito, S., *J. Endocrinol.*, 18, 245 (1959)
96. Greer, M. A., *Recent Progr. in Hormone Research*, 13, 67 (1957)
97. D'Angelo, S. A., *J. Endocrinol.*, 17, 286 (1958)
98. Reichlin, S., *Endocrinology*, 66, 340 (1960)
99. Bogdanove, E. M., and D'Angelo, S. A., *Endocrinology*, 64, 53 (1959)
100. Purves, H. D., *Symposium Lectures and Round Table Discussion, Intern. Congr. of Endocrinol., 1st Congr.*, 21 (Hamburger, C., Ed., Periodica, Copenhagen, Denmark, 1960)
101. Bottari, P., *Ciba Foundation Colloq. on Endocrinol.* 11, 52 (1957)
102. Crossan, J., Falch, J., and Reichlin, S., *Endocrinology*, 66, 777 (1960)
103. Ford, D. H., and Gross, J., *Endocrinology*, 63, 549 (1958)
104. Yamada, T., and Greer, M. A., *Endocrinology*, 64, 559 (1959)
105. Yamada, T., *Endocrinology*, 65, 216 (1959)
106. Yamada, T., *Endocrinology*, 65, 920 (1959)
107. Reichlin, S., *Endocrinology*, 66, 327 (1960)
108. Postel, S., *Endocrinology*, 58, 557 (1956)
109. McKenzie, J. M., *Endocrinology*, 63, 372 (1958)
110. Halmi, N. S., Granner, D. K., Albert, H., and Doughman, D. J., *Endocrinology*, 65, 101 (1959)
111. Rauschkolb, E. W., Farrell, G. L., and Koletsky, S., *Am. J. Physiol.*, 184, 55 (1956)
112. Davis, J. O., Bahn, R. C., Yankopoulos, N., Kliman, B., and Peterson, R. E., *Am. J. Physiol.*, 197, 380 (1959)
113. Ganong, W. F., Lieberman, A. H., Daily, W. J. R., Yuen, V. S., Mulrow, P. J., Luetscher, J. A., Jr., and Bailey, R. E., *Endocrinology*, 65, 18 (1959)
114. Davis, J. O., Bahn, R. C., and Ball, W. C., *Am. J. Physiol.*, 197, 387 (1959)
115. Farrell, G., *Physiol. Revs.*, 38, 709 (1958)
116. Farrell, G., *Recent Progr. in Hormone Research*, 15, 275 (1959)
117. Rauschkolb, E. W., and Farrell, G. L., *Endocrinology*, 59, 526 (1956)
118. Yankapoulos, N., Davis, J. O., Kli-

22. Mori, W., and Lerner, A. B., *Endocrinology*, 67, 443 (1960)
23. Bagnara, J. T., *Science*, 132, 1481 (1960)
24. Spirtos, B. N., and Halmi, N. S., *Endocrinology*, 65, 669 (1959)
25. Hertz, R., *Endocrinology*, 65, 926 (1959)
26. Etkin, W., and Lehrer, R., *Endocrinology*, 67, 457 (1960)
27. Rothchild, I., *Endocrinology*, 67, 9 (1960)
28. Haun, C. K., and Sawyer, C. H., *Endocrinology*, 67, 270 (1960)
29. Benson, G. K., and Folley, S. J., *J. Endocrinol.*, 16, 189 (1957)
30. McCann, S. M., Mack, R., and Gale, C., *Endocrinology*, 64, 870 (1959)
31. Rothchild, I., and Quilligan, E. J., *Endocrinology*, 67, 122 (1960)
32. Harris, G. W., *Neural Control of the Pituitary Gland* (Williams & Wilkins Co., Baltimore, Md., 1955)
33. Harris, G. W., *Symposium Lectures and Round Table Discussion, Intern. Congr. of Endocrinology, 1st Congr.*, 15 (Hamburger, C., Ed., Periodica, Copenhagen, Denmark, 1960)
34. Bush, I. E., and Mahesh, V. B., *J. Endocrinol.*, 18, 1 (1960)
35. Halberg, F., Peterson, R. E., and Silber, R. H., *Endocrinology*, 64, 222 (1959)
36. Halberg, F., Albrecht, P. G., and Bittner, J. F., *Am. J. Physiol.*, 197, 1083 (1959)
37. Anderson, E., Bates, R. W., Hawthorne, E., Haymaker, W., Knowlton, K., Rioch, D. M., Spence, W. T., and Wilson, H., *Recent Progr. in Hormone Research*, 13, 21 (1957)
38. Royce, P. C., and Sayers, G., *Endocrinology*, 63, 794 (1958)
39. Martini, L., Pecile, A., Saito, S., and Tani, F., *Endocrinology*, 66, 501 (1960)
40. Ganong, W. F., and Hume, D. M., *Endocrinology*, 55, 474 (1954)
41. Greer, M. A., and Erwin, H. L., *Endocrinology*, 58, 665 (1956)
42. McCann, S. M., *Endocrinology*, 60, 664 (1957)
43. Newman, A. E., Redgate, E. S., and Farrell, G., *Endocrinology*, 63, 723 (1958)
44. McCann, S. M., and Haberland, P., *Endocrinology*, 66, 217 (1960)
45. Mason, J. W., *Endocrinology*, 63, 403 (1958)
46. Suzuki, T., Romanoff, E. B., Koella, W. P., and Levy, C. K., *Am. J. Physiol.*, 198, 1312 (1960)
47. Mirsky, I. A., Stein, M., and Paulisch, G., *Endocrinology*, 55, 28 (1954)
48. McCann, S. M., Fruit, A., and Fulford, B. D., *Endocrinology*, 63, 29 (1958)
49. Casentini, S., De Poli, A., Hukovic, S., and Martini, L., *Endocrinology*, 64, 483 (1959)
50. Smelik, P. G., and De Wied, D., *Experientia*, 14, 17 (1958)
51. Hume, D. M., *Pathophysiologica Diencephalica* 217 (Curri, S. B., and Martini, L., Eds., Springer-Verlag, Vienna, Austria, 1958)
52. Kwaan, H. C., and Bartelstone, H. J., *Endocrinology*, 65, 982 (1959)
53. Martini, L., De Poli, A., Pecile, A., Saito, S., and Tani, F., *J. Endocrinol.*, 19, 164 (1959)
54. Royce, P. C., and Sayers, G., *Proc. Soc. Exptl. Biol. Med.*, 98, 70 (1958)
55. Hume, D. W., and Nelson, D. H., *Proc Endocrinol. Soc., 39th Meeting*, 98 (Charles C Thomas, Springfield, Ill., 1957)
56. Sayers, G., "Hormones in Blood," *Ciba Foundation Colloq. on Endocrinol.*, 11, 138 (1957)
57. Hilton, J. G., Scian, L. F., Westerman, C. D., Nakano, J., and Kruesi, O. R., *Endocrinology*, 67, 298 (1960)
58. Nichols, B., and Guilleman, R., *Endocrinology*, 64, 914 (1959)
59. Guilleman, R., Hearn, W. R., Cheek, W. R., and Householder, D. E., *Endocrinology*, 60, 488 (1957)
60. Royce, P. C., and Sayers, G., *Proc. Soc. Exptl. Biol. Med.*, 98, 677 (1958)
61. Rumsfield, H. W., and Porter, J. C., *Endocrinology*, 64, 942 (1959)
62. Nowell, N. W., *Endocrinology*, 64, 191 (1959)
63. Slusher, M. A., *Endocrinology*, 63, 412 (1958)
64. Egdahl, R. H., *Endocrinology*, 66, 200 (1960)
65. Mason, J. W., Nauta, W. J. H., Brady, J. W., and Robinson, J. A., *Proc. Endocrinol. Soc., 41st Meeting*, 29 (Charles C Thomas, Springfield, Ill., 1959)
66. Farrell, G., *Circulation*, 21, 1009 (1960)
67. Wurtman, R. J., Altschule, M. D., and Holmgren, U., *Am. J. Physiol.*, 197, 108 (1959)

PSYCHIATRY: PSYCHOMETRICS[1,2,3]

By C. L. Winder, Ph.D.

Department of Psychology, Stanford University, Stanford, California

This chapter is on selected aspects of the topic of psychological assessment, particularly as related to the medical specialty of psychiatry. The topics are chosen from among those that have become particularly relevant in the development and use of tests by clinical psychologists. Studies cited are intended only as examples because space limitations precluded any effort at full citation.

In practice and in empirical studies, a wide spectrum of frames of reference for the use of tests is found. To ignore these explicit or implicit frameworks is to neglect a major basis for identifying problems and progress.

One frame of reference starts with the assumption that there are mental illnesses and that patients are to be assigned to discrete disease categories. While much criticized, this approach is often found. Formal methodological problems are receiving long-needed consideration [e.g. (1, 2)]. A second frame of reference assumes that there are dimensions of psychopathology and the purpose of testing is to specify the position of each patient on these variables, or to predict course in terms of variables and treatments. The traditional psychometric methods are well adapted to this framework. A third frame of reference assumes that some comprehensive set of variables which subsumes psychopathology and personality can be invented. Diagnosis of an individual is accomplished when his position on each dimension is determined, usually by testing. Factor analysis methods are often applied although other methods are currently suggested [e.g. (3, 4)]. A fourth frame of reference involves some form of psychodynamic theory. Testing is to reveal *characteristic levels of various motivations, defenses, and coping* processes. A fifth frame of reference involves the element that psychological assessment of an individual must allow for the creation of concepts that apply to the one case. Tests, in a loose use of the term, are the basis for an inductive conceptualization of the subject, particularly of his uniqueness.

Tests and Psychiatric Diagnosis

Psychiatric diagnoses may be viewed as mutually exclusive categories, as terms implying a pattern of extremes of critical characteristics, or as

[1] The survey of the literature pertaining to this review was concluded in August, 1960.

[2] The following abbreviations are used: MMPI (Minnesota Multiphasic Personality Inventory); TAT (thematic apperception test).

[3] The term "psychometrics" is used simply to indicate the general topic of psychological testing.

man, B., and Peterson, R. E., *J. Clin. Invest.*, 38, 1278 (1959)
119. Mulrow, P. J., Shmagranoff, G. L., Lieberman, A. H., Slade, C. I., and Luetscher, J. A., Jr., *Endocrinology*, 64, 631 (1959)
120. Orti, E., and Ralli, E. P., *Am. J. Physiol.*, 199, 43 (1960)
121. Ross, E. J., and McLean, E. K., *Short Communications, Intern. Congr. Endocrinol. 1st Congr.*, 69 (Fuchs, F., Ed., Periodica, Copenhagen, Denmark, 1960)
122. Farrell, G., *Endocrinology*, 65, 29 (1959)
123. Farrell, G., *Endocrinology*, 65, 239 (1959)
124. Farrell, G., *Federation Proc.*, 19, 601 (1960)
125. Farrell, G., *Circulation*, 21, 1009 (1960)
126. Denton, D. A., Goding, J. R., and Wright, R. D., *Clinical Endocrinology*, 373 (Astwood, E. B., Ed., Grune & Stratton, Inc., New York, N. Y., 1960)
127. Denton, D. A., Goding, J. R., and Wright, R. D., *Brit. Med. J.*, II, 447 (1959)
128. Denton, D. A., Goding, J. R., and Wright, R. D., *Brit. Med J.*, II, 522 (1959)
129. Langley, L. L., Beall, W. A., and Smith, J. A., *Am. J Physiol.*, 197, 565 (1959)
130. Schneyer, C. A., and Schneyer, L. H., *Am. J. Physiol.*, 199, 55 (1960)
131. Davis, J. O., *Recent Progr. in Hormone Research* (In press, 1960)
132. Richards, J. B., and Sayers, G., *Proc. Soc. Exptl. Biol. Med.*, 77, 87 (1951)
133. Stein, O., and Gross, J., *Endocrinology*, 65, 707 (1959)
134. Crawford, J. D., and Pinkham, B., *Endocrinology*, 55, 699 (1954)
135. Smith, H. W., *Am. J. Med.*, 23, 623 (1957)
136. Peters, J. P., Welt, L. G., Sims, E. A. H., Orloff, J., and Needham, J., *Trans. Assoc. Am. Physicians*, 63, 57 (1950)
137. Wise, B. L., *Proc. Soc. Exptl. Biol. Med.*, 91, 557 (1956)
138. Wise, B. L., and Ganong, W. F., *Am. J. Physiol*, 198, 1291 (1960)
139. Gilbert, G. J., *Anat. Record*, 126, 2 (1956)
140. Gilbert, G. J., *Neurology*, 10, 138 (1960)
141. Farrell, G., and Travis, R. H. (Unpublished observations)
142. Barbarossa, C., De Martino, C., Peruzy, D., and Torlonia, G., *Advance Abstr. of Short Communications, Intern. Congr. of Endocrinol.*, 79 (Fuchs, F., Ed., Periodica, Copenhagen, Denmark, 1960)
143. Bartter, F. C., and Gann, D. S., *Circulation*, 21, 1016 (1960)
144. Gann, D. S., Mills, I. H., and Bartter, F. C., *Federation Proc.*, 19, 605 (1960)
145. Gann, D. S., Cruz, J. F., Casper, A. G. T., and Bartter, F. C., *Proc. Endocrine Soc., 42nd Meeting*, 14 (Charles C Thomas, Springfield, Ill., 1960)

occur together. There is legitimate debate as to the optimum method, but factor analysis is often favored. A relatively small number of descriptive variables which, together subsume all items, is derived. Every patient can then be assessed in terms of a set of scores on these variables.

Wittenborn (12) arrived at a system of nine variables after studying large samples of mental hospital patients. The variables are: Acute Anxiety, Conversion Hysteria, Manic State, Depressed State, Schizophrenic Excitement, Paranoid Condition, Paranoid Schizophrenia, Hebephrenic Schizophrenia, and Phobic Compulsive. Even though traditional sounding titles are used, the essential quality is that these are descriptive variables yielding a profile for each subject. Recently, Lorr, O'Connor & Stafford (3) analyzed a 172-item behavior inventory completed on 1400 psychotic patients in 47 hospitals. Four scales were extracted: Withdrawal, Thinking Disorganization, Agitated Depression, and Paranoid Belligerence. On the first three scales, open ward patients were rated less pathological than closed ward patients. Other projects of this type have been reported [e.g. (13)] as have more preliminary projects with outpatients (14, 15).

In principle, the variables need not be only descriptive. Wittenborn & Kline (16) suggest that different levels of drives may be associated with different descriptive variables, and offer relevant evidence. In general, as the experimental literature on psychopathology accumulates [e.g. (17)], items on physiological processes, psychological functioning, and life history would be incorporated into these systems. With refined systems, studies of etiology, course, and specific treatments might well be sharpened. Such variables would have advantages in validation of psychological tests.

The usual practical problems may well appear as the above approaches are used. For example, agreement between ratings made by nurses and other so-called ancillary personnel with those made by psychiatrists and psychologists may be poor (18).

Systems of personality variables.—Observations other than those deemed important in the standard nosology may be selected as the basis for developing a system of variables. And, the system may be developed on the general population and then used with patients. In certain respects, Cattell (19) is an example. The goal is to encompass all aspects of personality in a comprehensive system. Essentially, the procedure began with a reduction of 4500 trait terms to 42 initial variables. Three behavior media (behavior ratings, responses to questionnaires, and reactions in experimental situations) were sampled by tests and the data for each media reduced by factor analysis. Selection of factors to be retained depends on a complex system in which account is taken of studies within and across media. Cattell proposes systematic assessment in clinical research and practice in these terms. Assessment would be of ability, personality, and dynamic traits, supplemented by unique trait diagnosis of each subject. Such studies as those done by Karson (20) and Scheier, Cattell & Horn (21) give limited support to the proposal.

patterns of responses to be explained in terms of a dynamic theory. Knowledge of the reliability of diagnoses is necessary if test validity, as assessed directly or indirectly in terms of discriminations among diagnostic groups, is to be fully understood. As has often been the case in the use of tests in other fields, work in clinical psychology and psychiatry has seldom come to grips effectively with the need to know about one of the major factors involved, in this case the psychiatric diagnosis.

Goldfarb (5) has contributed to the meager literature on the reliability of diagnosis. Four clinical psychologists, working independently, diagnosed Veterans Administrations pension review cases. Categories used were: psychophysiologic reaction, psychoneurotic reaction, psychotic reaction, personality reaction, and brain damage (6). Typical pairs of judges agreed in 60 per cent of 100 cases. After a time interval, the typical judge agreed with himself in 60 per cent of his cases. In an earlier study (7), psychiatric residents and staff psychiatrists at a state hospital served as judges. Considering three major diagnostic categories, there was agreement in 84 per cent of 426 cases. The categories were: organic, psychotic, and character (psychoneurotic and personality reactions combined). Agreement on subcategories throughout the diagnostic system was low and would require improvement for research or practical purposes. Other studies are even less encouraging (8, 9).

Many recommend that the nosological system be abandoned as useless (10), but continued use of the terminology to designate samples and diagnostic groups as criteria in validation of tests is found throughout the literature. At the least, each investigator should take an explicit stand as to the implications he intends, and determine the reliability (interjudge agreement) within his project. If the standard diagnostic system is to be used, Orgel's (11) example might be followed. He has shown with refined definitions of paranoid and hebephrenic schizophrenia, very good agreement on these subcategories when the selection was made difficult by use of a large, heterogeneous sample.

Alternatives to Standard Diagnoses

Assuming (*a*) that, at best, standard diagnoses can be made with limited agreement; (*b*) that utility as a basis for specification of treatment and disposition is questionable; and (*c*) that heuristic value in studying etiology is low, it is not surprising that many alternatives are proposed.

Systems of descriptive variables.—A group of projects can be characterized by the fact that, in them, it is the nosological categories that are abandoned, not the signs, symptoms, and characteristics that have been employed in the study and evaluation of patients. Essentially, statements of signs and symptoms are collected, with efforts being made to be comprehensive. Large numbers of patients of all types are then observed and scored on these items. It is then possible to determine which items tend to

Those who would rely almost completely on factor analysis in identifying variables have claimed construct validation [e.g. (30)], but as yet the programs seem to be trait validation, not nomological validation.

Trait validation involves empirical demonstration that there is a low correlation of the test with measures of variables from which it is supposed to differ, as well as high correlation with an alternative measure of the same variable using a second method [see (31)].

Attention to the difference between attributing variance to content of test items, as distinct from attributing variance to the form of the items, has been minimal until recently. Earlier the "halo effect" in ratings and "apparatus factors" in laboratory work had been apparent. Now so-called response sets are receiving overdue attention. Examples, identified most clearly on inventories and questionnaires, are acquiescence, denial, taking extreme positions, and social desirability. To the extent that a response set is operating, the content of the items cannot be used as the basis for direct inference. The response sets can, of course, be taken as traits and may become the subject of psychological explanation. At present, interest seems to be focused on the problem which response sets represent in the development and utilization of inventory and other measures where it is the content implications of the items that is deemed primary (32). A rather extreme proposal regarding sets is that made by Berg (33), who suggests that item content is not important and proposes to focus exclusively on the set to give deviant responses.

Clinical Judgments

Since the power of theories of human behavior is low, and since the precision of measurement is not great, and since there are many variations in practice regarding such matters as treatment and disposition, work with patients involves a great number of judgments by clinicians. Meehl (34) focused the issue of the relative accuracy of actuarial prediction versus clinical (instrument plus interpreter) predictions. Holt (35) called attention to ways in which the problem had been simplified excessively. Nevertheless, consideration of relative accuracy, and analysis of performance has suggested improvements, both practical and theoretical.

Most studies of relative accuracy indicate either the superiority of actuarial prediction, or show equality (34, 36). Oskamp (36) emphasizes that in many studies clinical prediction has been handicapped because of limited amounts and kinds of information, because of unfamiliarity of judges with criteria or population, and because the accuracy of the actuarial system was calculated on the sample on which the empirical system was derived rather than a cross-validation sample. Oskamp's study confirms the conclusion that, given a fair evaluation, clinical does not exceed actuarial prediction in accuracy. Further studies are in order, but this conclusion will probably hold (37).

One criticism of studies of actuarial versus clinical prediction has been

But the adequacy of Cattell's published tests must be questioned and there are still serious problems regarding the development of the basic system [e.g. (22)].

The argument could be made that each theory of personality, in which stability is ascribed to major properties of the behaving organism, involves a system of variables that could guide any interested party in test development. Nowhere is this approximated.

Test Validation

In 1954, there was published by the American Psychological Association a statement regarding information that should accompany published tests (23). Among the proposed approaches in test validation was that called construct validation (the others were content, predictive, and concurrent validation). The approach was further elaborated by Cronbach & Meehl (24). Construct validation would take place within a formal theory. The form of the test, the items selected, the content of items, and the nature of the responses would be articulated with the theory [e.g. (25, 26)]. The proposal has been criticized, and recently Bechtoldt (27) has marshalled a strong argument against the continued use of the construct validation model on the grounds that by violating aspects of logical behaviorism the approach may ultimately lead to a non-empirical, non-scientific study of behavior. Campbell's answer (28) points to the need for the concept in the context of the testing movement in psychology and properly emphasizes that often the approach has not actually been followed, but only claimed. Sioce the term "theory" is often oot clearly defined or, if defined, tben the definition not ogreed upon, it has been possible to claim construct validation on a minimal basis.

Campbell proposes a further useful distinction. If the test is designed to measure a syndrome, trait, or variable (or some set of these) which is not part of a formal theory, tben the name "trait validity" is to be used. If the test performance is made the subject of clear predictions derived from a formal theory, then the name "nomological validity" is appropriate. This is in part simply a semantic matter. But there is the clear implication that before nomological validity is claimed, novel uses of the test must have been apparent from theory, and novel predictions must have been successful upon empirical check.

Campbell uses the Taylor Anxiety Scale as an example. When the test is validated against psychiatrists' ratings, trait validity is illustrated. But when the same test is treated as a measure of drive in Hull-Spence learning tbeory, nomological validation is exemplified. In the former, tbe inference is of a free-floating variable. In the latter, a formal construct is involved. Atkinson's (29) use of a thematic test as a measure of achievement need in combination with tbe Test Anxiety Questionnaire as a measure of the disposition to avoid failure, in the context of his theory of personality dynamics seems to qualify as nomological validation. Such examples are rare.

Scale which emphasizes heterosexual interest and adjustment as well as number and intimacy of friendships (44). History of psychopathology in the family background was not related to response to treatment, but among patients with no such family history, presence of relevant precipitating factors did relate to outcome (48). There is evidence that kind and extent of relationships of such life history variables to psychopathology varies with such factors as socioeconomic background [e.g. (49)]. In the development of prognostic instruments, the precision attained might well be greater if attention were given to groupings by aspects of family background.

A further basis for estimating prognosis is behavioral adjustment immediately after admission. For example, measures of motility, cooperation, and communication were found to be related to length of hospitalization (50).

More informed decisions regarding discharge would result if factors involved in posthospital adjustment were better understood. Amount of hospital treatment before and the type of ward from which the patient leaves are both related to success in foster home placement (51). In a similar study, trial visit success was related to a number of items, all of which seemed to reflect chronicity. Confidence in the results is greatly enhanced because base rate was taken into account and a cross-validation study was made (52). Prediction according to base rate would have been accurate in 60 per cent of the cases. On the cross-validation sample, the system was accurate in 84 per cent of cases. While this level of accuracy leaves something to be desired, it is high compared with most predictive procedures.

More specific predictions are attempted. For example, if the patient is given psychotherapy, how will he respond? Experience suggests that some will terminate soon after initiation of treatment. In an outpatient clinic, Hiler (53) found that prediction of early termination was slightly better from a sentence-completion test than from the Rorschach or Wechsler-Bellevue. On the cross-validation sample, the system resulted in 71 per cent correct predictions. That Rorschach performance is correlated with abrupt termination is further supported by Affleck & Mednick (54).

Poor response to extended, intensive psychotherapy by hospitalized patients is related to poor performance on the Wechsler-Bellevue and Rorschach. Good test performances were not prognostic (55).

Assuming that different treatments are specific for certain groups of patients, is clinical judgment an effective basis for assigning patients to treatments? The available results cast doubt on the accuracy of such judgments (56, 57).

Brain damage.—Williams, Lubin & Gieseking (58) studied a group of patients who had been tested before and after brain damage was sustained. This design permits a fundamental contribution to knowledge. Vocabulary is not resistant to the effects of brain damage, as has been supposed by many clinicians. There is no evidence of differential deficit on a variety of tests in that differential weighting did not improve the discrimination between organics and controls.

The Bender Gestalt test permits better than chance identification of

that the task was not a global personality assessment. That global assessment has some validity when done by experts is demonstrable [e.g. (38, 39)]. The disciplined development of this ability is socially valuable since much clinical work would permit development of an actuarial system only very gradually. But, if an actuarial system is feasible, it will be as accurate and probably more economical, and will make identification of errors more certain. When clinical judgment seems necessary, then the clinician should be validated.

The investigation of clinical judgment has gone beyond the above issues. Appropriateness of confidence and effects of specific training have had an initial exploration (36). And, a rigorous model for analysis of the rating behavior of judges has been developed by Hoffman (40). Already it is clear that while clinical judgment is configural, the departure from a simple linear procedure is small.

Whether judgment is actuarial or clinical, accuracy of the prediction of a dichotomous criterion using any specific cutting point is a function of the frequency of occurrence of various types of patients in a given sample. Thus, the accuracy of assessment can change simply because the so-called base rate (41) is different from time to time and place to place. There is reason to believe that the diligent and truly expert clinician may be able to meet even the challenge of base rates (42).

Some Criterion-Oriented Studies

Prognosis.—The Rorschach test has been used widely in prediction of such criteria as length of hospitalization. One issue here is that of the atomistic use of specific signs versus global assessment. The rise and fall of various atomistic signs has raised doubts regarding the Rorschach having any validity for prognosis. Zamansky & Goldman (43) report superiority of global clinical assessment. Developmental level scoring of the Rorschach has proved somewhat valid for various purposes, and has been applied with some success in estimating the adjustment of hospitalized patients, as has Klopfer's Prognostic Rating Scale (44). Length of hospitalization was not predicted by features of performance on the Bender Gestalt (45). If newly admitted patients took the Minnesota Multiphasic Personality Inventory in the usual way and then attempted to simulate "the normal" performance, earlier hospital discharge was related to relative responsiveness to these instructions to simulate (46).

Systematic use of information regarding life history, adjustment, and psychopathology seems more promising than widely used tests in estimating prognosis. This is not to say that this would hold when information is used in the standard clinical, impressionistic manner. Over-all, experience suggests that organization of the material into formal scales eventuates in greater precision. Marital status, degree of incapacity, diagnosis, legal competence, and use of alcohol relate to length of hospitalization (47) in one study, which included cross-validation. Long-term prognosis of schizophrenics is somewhat predictable from Section I of the Phillips Prognostic Rating

hension tests (80) appear to be correlated with the severity continuum. The Rorschach scored for developmental level has now been validated by a number of studies, e.g., as related to open versus closed ward placement and amount of social participation in situations requiring cooperation (81).

A recognition test of vocabulary probably gives a better estimate of premorbid intelligence than does a recall test (82). It is on the latter type that deteriorated patients do very poorly.

Psychoneurotic reactions.—In addition to the studies mentioned above, there seem to be a few which focus on the identification of neurotics. Several studies report on outpatient groups but do not find distinctive results for the subgroup of neurotics. There are some continuing reports that manifest anxiety, as measured by questionnaires, is high among outpatients (83). The possibility that schizophrenics tend to respond with positive extremes and neurotics with negative extremes is suggested [e.g. (84)]. A procedure which permits fairly adequate discrimination between psychotics and neurotics involves the classical laboratoy methods of delayed reaction, alternation, and serial reaction tasks (85). Furthermore, the authors provide considerable information on the effects of age and intelligence.

Psychopathic personality.—Here are grouped some studies of delinquents and criminals. The old issue as to whether Performance IQ exceeds Verbal IQ in such cases is continued (86, 87, 88).

For the assessment of position on a socialization continuum, Gough (89) has developed a very effective inventory scale. While the scale would undoubtedly prove to be heterogeneous under factor analysis, it is impressive. Not including the initial studies, 41 research samples are reported. These included 1295 male and 784 female delinquents, and 9001 male and 9776 female non-delinquents. This increases confidence that the scale can be used in a variety of settings without the usual radical fluctuations in the distribution of scores. The scale does not, as it should not, yield differences for Negro and white delinquents, nor for large city versus other area delinquents. There is no correlation between the socialization score and the area of residence in a large city (90).

Miscellaneous.—Of course, there are many more studies than can be mentioned in this chapter. Identification of homosexuals seems to sustain interest, with some success reported for the Rorschach and TAT systems (91, 92). There are impressive case studies which illustrate clinical judgment and formulation [e.g. (93)]. A multitude of MMPI scales for diagnostic use are constantly produced (94), often with no consideration of overlap or effectiveness compared with existing scales. Four groups of patients representing increasing degrees of psychiatric disturbance were ordered in the expected way by the Cornell Medical Index (95). Many new tests are published but few, if any, are unusually accurate.

Some Test-Oriented Studies

Rorschach.—Baughman (96) has reported on his extensive empirical study of the effects of systematic changes in the stimulus cards. There is

organics, especially if the number of organics and non-organics is about equal (59). Objective scoring and global judgment are about equally valid (60). If extreme cases are not considered, validity is questionable (60). The spiral aftereffect test seems more effective in predicting abnormal EEG findings among children than does the Bender Gestalt, Draw-a-Person, or Wechsler Intelligence Scale for Children scales (61). Validity of the spiral test for adults is again questioned (62). Flicker fusion frequency (59), the Halstead Impairment Index (63), and several other techniques [e.g. (64 to 67)] have some power in the identification of organics. Assessment of memory impairment may require several tests that depend on different systems (68). The Trail-Making Test seems to be sensitive to hemisphere damage in addition to contributing in differentiating organics from normals (69). Organics with more actively interfering physiological processes had more of Piotrowski's signs on the Rorschach (70).

Schizophrenic reactions.—There continues to be a large number of projects in which the discriminatory power of tests in separating normals and schizophrenics is studied. Frequently, such studies involve an effort to utilize a specific hypothesis. Thus, not only do schizophrenics make inferior interpretations of proverbs, but one investigator reports that oral proverbs cause more deficit than do anal or phallic proverbs (71). Usually, such variables as item difficulty receive no consideration in these studies.

Loy (72) has checked the application of the Taulbee-Sission MMPI system to female patients, rather than simply assuming that it applies. Selection was less successful for female neurotics but somewhat more successful for female schizophrenics. To differentiate schizophrenics from neurotic and personality disorder cases, Eichman (73) proposes another MMPI scale. Such comparison groups make the results relevant, at least, to the problem of differential diagnosis. Mean scores of groups of normals, neurotics, and schizophrenics differ if the Thematic Apperception Test is scored by a system involving notation of the extent to which usual elements of the response to each card are present (74). Kataguchi (75) reports on his Rorschach Schizophrenic Score. With equal numbers of normals, neurotics, and schizophrenics, the recommended cut-off point selected 77 per cent of the schizophrenics, 13 per cent of the neurotics, and none of the normals. This is an unusually effective discrimination, compared with other approaches and tests. Weckowicz (76) found varying levels of proficiency on a hidden pictures test among controls (normals and non-schizophrenic, non-organic psychiatric patients), acute schizophrenics, chronic schizophrenics, and organics, which seems to be the minimum that should be expected of a diagnostic instrument. In an examination of MMPI paranoid scale "hits" and "misses," it was found that test-taking attitude or set may help explain the errors (77).

With increasing evidence that schizophrenics differ markedly in severity [e.g. (17)], some interest has developed in the sensitivity of tests to this factor. A developmental level score on the Rorschach (78), performance on proverbs (79), and extent of disorganization on vocabulary and compre-

fulness as indices of any particular style" [(109), p. 249]. Investigators are showing ingenuity in clarifying the varieties and effects of response styles in MMPI results (110, 111). Instruments developed to measure response styles may take a place in diagnostic assessment because some studies find that diagnostic groups differ in predominant styles (112). Care in item construction may be the best means of reducing unwanted response style effects [e.g. (113)]. The vital questions regarding the psychology of response styles need much clarification, and some work has been reported (114). Clinical uses of the MMPI should take account of these developments.

In Conclusion

Tests do not have uniform validity for all groups and the relevant parameters are often ignored, but sometimes the relevant parameters are not known. For example, predictions seem to differ in accuracy for groups that respond differently to stress (115). There are many reasons for checking most instruments for accuracy in each setting, even though this is expensive and inconvenient.

Continuous efforts are necessary to make an examiner standard, as well as the test. Warmth of the subject while he is being tested affects stringency of scoring, amount of encouragement given the subject, and the number of opportunities given the subject to clarify or correct responses (116).

In test interpretation, descriptive variables tend to be used as though they were explanatory concepts (117). And progress toward solution of the problem of communication of results and interpretations of assessments is urgently needed (118).

Advances in the understanding of psychopathology suggest new assessment procedures such as the systematic use of interviews (119), measures of the amounts of disorganization related to different types of interpersonal relationships (120), and measures of aspects of functioning such as maintenance of perceptual constancy (121).

Continued intensive study of widely used tests gives more and more evidence of the limited validity of these instruments. In part, this is due to defective criteria and indefinite conceptualizations. But, also, it reflects limitations of the test situations in contrast to the wide range of stimuli to which humans respond selectively, and the wide range of responses made. Systematic observation of subjects has not been developed as an assessment technique in proportion to its promise. This would appear to be the most appropriate direction in development of new techniques for use in psychiatry and clinical psychology. Repeatedly, it is assessment based on observations which proves to be valid in terms of behavioral criteria (122).

Finally, as time passes, each individual changes. Assessment tends to emphasize stability. Thus, the emerging adaptation to the new situation, or simply to subsequent events limits the precision with which predictions can be made.

clear evidence of the importance of structural aspects of the blots in addition to form. A new approach to the study of color shock appears in a study by Wolpin & Hamlin (97) who found that incongruity of form and color is associated with decreased popular responses and increased use of color. Meaning of the inkblots has been approached through the use of the semantic differential (98), and among other things there is some support for the father-mother interpretation of cards IV and VII (99). Fortunately, some experimental work is being done on the relationship between experience and responses to ambiguous stimuli (100).

The hypothesis that anxiety can be inferred from shading responses is given mild support (101), but this may be more valid for psychotics than others (102). Type of paranoid delusion is related to type of human movement response, as predicted (103). Instructions that certain topics be avoided is followed by an increase in inanimate movement responses (104). These and other projects intended to enhance understanding of the determinant categories are not convincing, but the search for appropriate evaluations of interpretative hypotheses is vital so long as conventional clinical use is made of this technique.

Normative data are still inadequate, and various aspects of administration and scoring are not carried out in a standardized manner, but the use of the Rorschach is defended on the basis of clinical experience [e.g. (105)].

Thematic apperception test (TAT).—Employing a complex and appropriate design, Henry & Farley (38) find evidence for rather global personality appraisal based on the TAT. The personality formulations were centered on peer relations, family dynamics, mental functioning, and emotional adjustment. Subjects were normal adolescents. Interpretations were made within one conceptual framework.

Several investigators propose that if thematic items of relatively low ambiguity were organized into scales, assessment would be improved. Little (106) offers a discussion of this proposal. Kagan (107) found stability of traits over long periods of time only if more unambiguous stimuli were utilized. Prediction of overt behavior from the TAT or other tests involving thematic items usually will require assessment of external and internal controls [e.g. (29, 108)]. There is often a lack of clarity as to just what is to be assessed by tests and this development of specific scales should sharpen the focus in the case of thematic tests.

Minnesota Multiphasic Personality Inventory (MMPI).—Many studies of the MMPI and other inventory techniques have focused on response style (response bias, response set). Traditionally, the assumption has been that responses are determined primarily by item content, but now it is clearly recognized that responses can be viewed as indicators of style, e.g., acquiescence. In the enthusiasm with this distinction, it appears that the comment of Jackson & Messick is well taken: "Little thought is given to the fact that these measures (such as the MMPI) may not only contain several dimensions of content, but of style as well, thus limiting their use-

fulness as indices of any particular style" [(109), p. 249]. Investigators are showing ingenuity in clarifying the varieties and effects of response styles in MMPI results (110, 111). Instruments developed to measure response styles may take a place in diagnostic assessment because some studies find that diagnostic groups differ in predominant styles (112). Care in item construction may be the best means of reducing unwanted response style effects [e.g. (113)]. The vital questions regarding the psychology of response styles need much clarification, and some work has been reported (114). Clinical uses of the MMPI should take account of these developments.

In Conclusion

Tests do not have uniform validity for all groups and the relevant parameters are often ignored, but sometimes the relevant parameters are not known. For example, predictions seem to differ in accuracy for groups that respond differently to stress (115). There are many reasons for checking most instruments for accuracy in each setting, even though this is expensive and inconvenient.

Continuous efforts are necessary to make an examiner standard, as well as the test. Warmth of the subject while he is being tested affects stringency of scoring, amount of encouragement given the subject, and the number of opportunities given the subject to clarify or correct responses (116).

In test interpretation, descriptive variables tend to be used as though they were explanatory concepts (117). And progress toward solution of the problem of communication of results and interpretations of assessments is urgently needed (118).

Advances in the understanding of psychopathology suggest new assessment procedures such as the systematic use of interviews (119), measures of the amounts of disorganization related to different types of interpersonal relationships (120), and measures of aspects of functioning such as maintenance of perceptual constancy (121).

Continued intensive study of widely used tests gives more and more evidence of the limited validity of these instruments. In part, this is due to defective criteria and indefinite conceptualizations. But, also, it reflects limitations of the test situations in contrast to the wide range of stimuli to which humans respond selectively, and the wide range of responses made. Systematic observation of subjects has not been developed as an assessment technique in proportion to its promise. This would appear to be the most appropriate direction in development of new techniques for use in psychiatry and clinical psychology. Repeatedly, it is assessment based on observations which proves to be valid in terms of behavioral criteria (122).

Finally, as time passes, each individual changes. Assessment tends to emphasize stability. Thus, the emerging adaptation to the new situation, or simply to subsequent events limits the precision with which predictions can be made.

LITERATURE CITED

1. McQuitty, L. L., In Bass, B. M., and Berg, I. A., Eds., *Objective Approaches to Personality Assessment*, 66–81 (D. Van Nostrand Co., Inc., Princeton, New Jersey, 1959)
2. Ledley, R. S., and Lusted, L. B., *Science*, 130, 9–21 (1959)
3. Lorr, M., O'Connor, J. P., and Stafford, J. W., *J. Clin. Psychol.*, 16, 241–45 (1960)
4. Eysenck, S. B. G., Eysenck, H. J., and Claridge, G., *J. Mental Sci.*, 106, 581–89 (1960)
5. Goldfarb, A., *J. Clin. Psychol.*, 15, 392–96 (1959)
6. *Diagnostic and Statistical Manual: Mental Disorders* (Am. Psychiatric Assoc., Washington, D. C., 1952)
7. Schmidt, H. O., and Fonda, C. P., *J. Abnormal Social Psychol.*, 52, 262–67 (1956)
8. Ash, P., *J. Abnormal Social Psychol.*, 44, 272–76 (1949)
9. Mehlman, B., *J. Abnormal Social Psychol.*, 47, 577–78 (1952)
10. Roe, A., *J. Abnormal Social Psychol.*, 44, 36–41 (1949)
11. Orgel, S. A., *J. Clin. Psychol.*, 13, 159–61 (1957)
12. Wittenborn, J. R., *Wittenborn Psychiatric Rating Scales* (Psychol. Corp., New York, N. Y., 1955)
13. Guertin, W. H., and Krugman, A. D., *J. Clin. Psychol.*, 15, 32–36 (1959)
14. Lorr, M., and Rubinstein, E. A., *J. Abnormal Social Psychol.*, 51, 514–22 (1955)
15. Tatom, M. H., *J. Consulting Psychol.*, 22, 73–81 (1958)
16. Wittenborn, J. R., and Kline, N. S., *J. Abnormal Social Psychol.*, 58, 300–4 (1959)
17. Winder, C. L., In Jackson, D. D., Ed., *The Etiology of Schizophrenia*, 191–247 (Basic Books, Inc., New York, N. Y., 1960)
18. Jensen, M. B., and Morris, W. E., *J. Clin. Psychol.*, 16, 248–52 (1960)
19. Cattell, R. B., *Personality and Motivation Structure and Measurement* (World Book Co., Yonkers-on-Hudson, New York, 1957)
20. Karson, S., *J. Clin. Psychol.*, 15, 174–76 (1959)
21. Scheier, I. H., Cattell, R. B., and Horn, J. L., *J. Clin. Psychol.*, 16, 135–45 (1960)
22. Becker, W. C., *Psychol. Bull.*, 57, 201–12 (1960)
23. *Technical Recommendations for Tests and Diagnostic Techniques* (Am. Psychology Assoc., Washington, D. C., 1954)
24. Cronbach, L. J., and Meehl, P. E., *Psychol. Bull.*, 52, 281–302 (1955)
25. Jessor, R., and Hammond, K. R., *Psychol. Bull.*, 54, 161–70 (1957)
26. Loevinger, J., *Psychol. Reports*, 3, 635–94 (1957)
27. Bechtoldt, H. P., *Am. Psychologist*, 14, 619–29 (1959)
28. Campbell, D. T., *Am. Psychologist*, 15, 546–53 (1960)
29. Atkinson, J. W., *Ann. Rev. Psychol.*, 11, 255–81 (1960)
30. Cattell, R. B., *J. Clin. Psychol.*, 13, 221–33 (1957)
31. Campbell, D. T., and Fiske, D. W., *Psychol. Bull.*, 56, 81–105 (1959)
32. Loevinger, J., *Ann. Rev. Psychol.*, 10, 287–316 (1959)
33. Berg, I. A., In Bass, B. M., and Berg, I. A., Eds., *Objective Approaches to Personality Assessment* (D. Van Nostrand Co., Inc., Princeton, New Jersey, 1959)
34. Meehl, P. E., *Clinical versus Statistical Prediction* (Univ. Minnesota Press, Minneapolis, Minn., 1954)
35. Holt, R. R., *J. Abnormal Social Psychol.*, 56, 1–12 (1958)
36. Oskamp, S., *The Relationship of Clinical Experience and Training Methods to Several Criteria of Clinical Prediction* (Unpublished dissertation, Stanford Univ., Stanford, Calif., 1960)
37. Meehl, P. E., *Am. Psychologist*, 15, 19–27 (1960)
38. Henry, W. E., and Farley, J., *J. Projective Techniques*, 23, 273–77 (1959)
39. Beck, S. J., *The Rorschach Experiment: Ventures in Blind Diagnosis* (Grune & Stratton, Inc., New York, N. Y., 1960)
40. Hoffman, P. J., *Psychol. Bull.*, 57, 116–31 (1960)
41. Meehl, P. E., and Rosen, A., *Psychol. Bull.*, 52, 194–216 (1955)
42. Goldberg, L. R., *J. Consulting Psychol.*, 23, 25–33 (1959)
43. Zamansky, H. S., and Goldman, A. E., *J. Projective Techniques*, 24, 75–82 (1960)
44. Seidel, C., *J. Consulting Psychol.*, 24, 46–49 (1960)
45. Higbee, D. S., Clarke, J. R., and

Henderson, W. E., *J. Clin. Psychol.*, 16, 265–66 (1960)
46. Grayson, H. M., and Olinger, L. B., *J. Consulting Psychol.*, 21, 73–77 (1957)
47. Lindemann, J. E., Fairweather, G. W., Stone, G. B., and Smith, R. S., *J. Consulting Psychol.*, 23, 85–89 (1959)
48. Scherer, I. W., and Nidorf. L. J., *J. Clin. Psychol.*, 16, 60–62 (1960)
49. Lane, R. C., and Singer, J. L., *J. Abnormal Social Psychol.*, 59, 328–39 (1959)
50. Ellsworth, R. B., and Clayton, W. H., *J. Consulting Psychol.*, 23, 15–20 (1959)
51. Ullmann, L. P., and Berkman, V. C., *J. Clin. Psychol.*, 15, 28–31 (1959)
52. Pishkin, V., and Bradshaw, F. J., Jr., *J. Clin. Psychol.*, 16, 85–88 (1960)
53. Hiler, E. W., *J. Consulting Psychol.*, 23, 544–49 (1959)
54. Affleck, D. C., and Mednick, S. A., *J. Consulting Psychol.*, 23, 125–28 (1959)
55. Rioch, M. J., and Lubin, A., *J. Consulting Psychol.*, 23, 313–18 (1959)
56. Garetz, F. K., Kogl, R. C., and Wiener, D. N., *J. Clin. Psychol.*, 15, 401–2 (1959)
57. Weiner, D. N., and Raths, O. N., Jr., *Am. J. Orthopsychiat.*, 29, 350–56 (1959)
58. Williams, H. L., Lubin, A., and Gieseking, C. F., *J. Consulting Psychol.*, 23, 300–5 (1959)
59. McGuire, F. L., *J. Clin. Psychol.*, 16, 276–78 (1960)
60. Nadler, E. B., Fink, S. L., Shontz, F. C., and Brink, R. W., *J. Clin. Psychol.*, 15, 39–41 (1959)
61. Blau, T. H., and Schaffer, R. E., *J. Consulting Psychol.*, 24, 35–42 (1960)
62. Philbrick, E. B., *J. Consulting Psychol.*, 23, 39–43 (1959)
63. Reitan, R. M., *J. Clin. Psychol.*, 15, 281–85 (1959)
64. Leventhal, D. B., McGaughran, L. S., and Moran, L. J., *J. Abnormal Social Psychol.*, 58, 84–90 (1959)
65. Cobrink, L., *Am. J. Psychol.*, 72, 566–71 (1959)
66. Butz, G. K., Hunsicker, A. L., and Hurd, D. E., *J. Clin. Psychol.*, 15, 274–80 (1959)
67. Shapiro, M. B., Kessell, R., and Maxwell, A. E., *J. Clin. Psychol.*, 16, 266–71 (1960)
68. Heilbrun, A. B., Jr., *J. Mental Sci.*, 106, 241–45 (1960)
69. Reitan, R. M., and Tarshes, E. L., *J. Nervous Mental Disease*, 129, 257–62 (1959)
70. Birch, H. G., and Diller, L., *J. Projective Techniques*, 23, 184–97 (1959)
71. Lewis, J. M., Griffith, E. C., Riedel, A. F., and Simmons, B. A., *J. Nervous Mental Disease*, 129, 564–67 (1959)
72. Loy, D. L., *J. Clin. Psychol.*, 15, 306–7 (1959)
73. Eichman, W. J., *J. Consulting Psychol.*, 23, 442–47 (1959)
74. Dana, R. H., *J. Projective Techniques*, 23, 307–10 (1959)
75. Kataguchi, Y., *J. Projective Techniques*, 23, 214–22 (1959)
76. Weckowicz, T. E., *Arch. Gen. Psychiat.*, 2, 521–27 (1960)
77. Kleinmuntz, B., *J. Clin. Psychol.*, 16, 310–12 (1960)
78. Friedman, H., *J. Clin. Psychol.*, 16, 52–54 (1960)
79. Becker, W. C., *J. Abnormal Social Psychol.*, 53, 229–36 (1956)
80. Jones, N. F., *J. Clin. Psychol.*, 15, 396–400 (1959)
81. Wilensky, H., *J. Projective Techniques*, 23, 87–92 (1959)
82. Blatt, S. J., *Arch. Gen. Psychiat.*, 1, 473–76 (1959)
83. Bailey, M. A., Berrick, M. E., Lachmann, F. M., and Ortmeyer, D. H., *J. Clin. Psychol.*, 16, 209–10 (1960)
84. Brengelman, J. C., *J. Mental Sci.*, 106, 187–92 (1960)
85. Pascal, G. R., and Jenkins, W. O., *J. Clin. Psychol.*, 15, 159–63 (1959)
86. Wiens, A. N., Matarazzo, J. D., and Gaver, K. D., *J. Clin. Psychol.*, 15, 191–93 (1959)
87. Foster, A. L., *J. Clin. Psychol.*, 15, 78–79 (1959)
88. Field, J. G., *J. Clin. Psychol.*, 16, 321–22 (1960)
89. Gough, H. G., *J. Consulting Psychol.*, 24, 23–30 (1960)
90. Peterson, D. R., Quay, H. C., and Anderson, A. C., *J. Consulting Psychol.*, 23, 182 (1959)
91. Yamahiro, R. S., and Griffith, R. M., *J. Clin. Psychol.*, 16, 21–24 (1960)
92. Lindzey, G., Tejessy, C., and Zamansky, H. S., *J. Abnormal Social Psychol.*, 57, 67–75 (1958)
93. Swartz, M., and Ferguson, E. D., *J. Clin. Psychol.*, 15, 124–27 (1959)
94. Panton, J. H., *J. Clin. Psychol.*, 15, 196–97 (1959)

95. Lawton, M. P., *J. Consulting Psychol.*, 23, 352–56 (1959)
96. Baughman, E. E., *J. Projective Techniques*, 23, 134–83 (1959)
97. Wolpin, M., and Hamlin, R. M., *J. Clin. Psychol.*, 15, 151–55 (1959)
98. Rabin, A. I., *J. Consulting Psychol.*, 23, 368–72 (1959)
99. Kamano, D. K., *J. Clin. Psychol.*, 16, 50–52 (1960)
100. Binder, A., and Feldman, S. E., *Psychol. Monographs*, 74, No. 9 (Whole No. 496) (1960)
101. Tong, J. E., and Murphy, I. C., *J. Clin. Psychol.*, 16, 324–28 (1960)
102. Ainsworth, M. D., and Kuethe, J. L., *J. Projective Techniques*, 23, 391–402 (1959)
103. King, G. F., *J. Projective Techniques*, 24, 161–63 (1960)
104. Neel, A. F., *J. Consulting Psychol.*, 24, 224–30 (1960)
105. Hertz, M. R., *J. Projective Techniques*, 23, 33–48 (1959)
106. Little, K. B., *J. Projective Techniques*, 23, 287–90 (1959)
107. Kagan, J., *J. Consulting Psychol.*, 23, 266–71 (1959)
108. Lesser, G. S., *J. Consulting Psychol.*, 23, 60–65 (1959)
109. Jackson, D. N., and Messick, S., *Psychol. Bull.*, 55, 243–52 (1958)
110. Edwards, A. L., *The Social Desirability Variable in Assessment and Research* (Dryden Publ. Co., New York, N. Y., 1957)
111. Wiggins, J. S., *J. Consulting Psychol.*, 23, 419–27 (1959)
112. Barnes, E. H., *J. Consulting Psychol.*, 20, 419–21 (1956)
113. Buss, A. H., *J. Consulting Psychol.*, 23, 510–13 (1959)
114. Couch, A., and Keniston, K., *J. Abnormal Social Psychol.*, 60, 151–74 (1960)
115. Fulkerson, S. C., *J. Clin. Psychol.*, 15, 169–73 (1959)
116. Masling, J., *J. Consulting Psychol* , 23, 336–41 (1959)
117. Liverant, S., *J. Consulting Psychol.*, 24, 101–10 (1960)
118. Bellak, L., *J. Nervous Mental Disease*, 129, 76–77 (1959)
119. Chapple, E. D., Chapple, M. F., Wood, L. A , Miklowitz, A., Kline, N. S , and Saunders, J. C., *Arch. Gen. Psychiat* , 3, 160–67 (1960)
120. Heath, D. H., *J. Gen. Psychol.*, 62, 165–76 (1960)
121. Weckowicz, T. E., and Blewett, D. B., *J. Mental, Sci.*, 105, 909–34 (1959)
122. Forsyth, R. P., and Fairweather, G. W., *J. Abnormal Social Psychol.* (In press)

PSYCHOTHERAPEUTIC DRUGS[1]

By B. B. Brodie, Ph.D., F. Sulser, M.D., and E. Costa, M.D.
Laboratory of Chemical Pharmacology, National Heart Institute, Bethesda, Maryland

A major goal of psychopharmacology is to explain how drugs alter mental states by modifying neurophysiological and biochemical processes in the central nervous system. This goal is still far distant, for we cannot yet describe how drugs affect gross neural pathways, much less their subtle effects on discrete neurons. It is the purpose of this review to describe pharmacological, neurophysiological, and biochemical actions of psychotherapeutic drugs that might contribute to our understanding of their clinical effects.

With respect to the peripheral nervous system, much evidence shows that drugs modify the transmission of nerve impulses by interfering with the action of acetylcholine or norepinephrine at various synapses. A drug can modify transmission by (*a*) mimicking a neurohormone, (*b*) blocking its action, (*c*) preventing its inactivation, (*d*) depleting it from nerve endings, (*e*) blocking its synthesis, or (*f*) preventing its physiological release. So specific are some drugs in their action that they literally "map out" the peripheral nervous system: muscarine mimics faithfully the action of acetylcholine at parasympathetic sites; and curare differentially blocks the oction of acetylcholine on myoneural junctions.

In the face of overwhelming evidence that drugs at the peripheral nervous system act by modifying synaptic transmission, many investigators ore still loathe to assume that psychotherapeutic agents might act centrally in o similar way; instead, numerous workers still entertain the possibility that drugs influence behavior by affecting processes such as oxidative phosphorylation or carbohydrate metabolism. But it does not seem logical that drugs would exert unique effects on behavior by influencing processes so universal in nature that they occur even in plants; it seems more realistic to believe that these drugs affect the unique biochemical processes involved in synaptic transmission.

Among the trials that beset the study of drug actions in the central nervous system is the difficulty of isolating functional units. The central nervous system is a mass of intercommunicating neurons; one nerve cell affects innumerable others, and even large functional units influence one another. This makes it almost impossible to assign a drug effect, electrical or biochemical, to a particular brain site; depression induced in one part of the brain might reflect a direct action of the drug, or an indirect action, owing to loss of normal stimulating inflow. Another stumbling block existed as long as only one neurohormone, acetylcholine, was defined in the central nervous system. The discoveries in recent years of other potential neurohormones in

[1] The survey of the literature pertaining to this review was concluded in September, 1960.

95. Lawton, M. P., *J. Consulting Psychol.*, 23, 352–56 (1959)
96. Baughman, E. E., *J. Projective Techniques*, 23, 134–83 (1959)
97. Wolpin, M., and Hamlin, R. M., *J. Clin. Psychol.*, 15, 151–55 (1959)
98. Rabin, A. I., *J. Consulting Psychol.*, 23, 368–72 (1959)
99. Kamano, D. K., *J. Clin. Psychol.*, 16, 50–52 (1960)
100. Binder, A., and Feldman, S. E., *Psychol. Monographs*, 74, No. 9 (Whole No. 496) (1960)
101. Tong, J. E., and Murphy, I. C., *J. Clin. Psychol.*, 16, 324–28 (1960)
102. Ainsworth, M. D., and Kuethe, J. L., *J. Projective Techniques*, 23, 391–402 (1959)
103. King, G. F., *J. Projective Techniques*, 24, 161–63 (1960)
104. Neel, A. F., *J. Consulting Psychol.*, 24, 224–30 (1960)
105. Hertz, M. R., *J. Projective Techniques*, 23, 33–48 (1959)
106. Little, K. B., *J. Projective Techniques*, 23, 287–90 (1959)
107. Kagan, J., *J. Consulting Psychol.*, 23, 266–71 (1959)
108. Lesser, G. S., *J. Consulting Psychol.*, 23, 60–65 (1959)
109. Jackson, D. N., and Messick, S., *Psychol. Bull.*, 55, 243–52 (1958)
110. Edwards, A. L., *The Social Desirability Variable in Assessment and Research* (Dryden Publ. Co., New York, N. Y., 1957)
111. Wiggins, J. S., *J. Consulting Psychol.*, 23, 419–27 (1959)
112. Barnes, E. H., *J. Consulting Psychol.*, 20, 419–21 (1956)
113. Buss, A. H., *J. Consulting Psychol.*, 23, 510–13 (1959)
114. Couch, A., and Keniston, K., *J. Abnormal Social Psychol.*, 60, 151–74 (1960)
115. Fulkerson, S. C., *J. Clin. Psychol.*, 15, 169–73 (1959)
116. Masling, J., *J. Consulting Psychol.*, 23, 336–41 (1959)
117. Liverant, S., *J. Consulting Psychol.*, 24, 101–10 (1960)
118. Bellak, L., *J. Nervous Mental Disease*, 129, 76–77 (1959)
119. Chapple, E. D., Chapple, M. F., Wood, L. A., Miklowitz, A., Kline, N. S., and Saunders, J. C., *Arch. Gen Psychiat.*, 3, 160–67 (1960)
120. Heath, D. H., *J. Gen. Psychol.*, 62, 165–76 (1960)
121. Weckowicz, T. E., and Blewett, D. B., *J Mental, Sci*, 105, 909–34 (1959)
122. Forsyth, R. P., and Fairweather, G. W., *J. Abnormal Sociol Psychol.* (In press)

PSYCHOTHERAPEUTIC DRUGS[1]

By B. B. Brodie, Ph.D., F. Sulser, M.D., and E. Costa, M.D.
Laboratory of Chemical Pharmacology, National Heart Institute, Bethesda, Maryland

A major goal of psychopharmacology is to explain how drugs alter mental states by modifying neuropbysiological and biochemical processes in the central nervous system. This goal is still far distant, for we cannot yet describe how drugs affect gross neural pathways, much less their subtle effects on discrete neurons. It is the purpose of this review to describe pharmacological, neurophysiological, and biochemical actions of psychotherapeutic drugs that might contribute to our understanding of their clinical effects.

With respect to the peripheral nervous system, much evidence shows that drugs modify the transmission of nerve impulses by interfering with the action of acetylcholine or norepinephrine at various synapses. A drug can modify transmission by (*a*) mimicking a neurohormone, (*b*) blocking its action, (*c*) preventing its inactivation, (*d*) depleting it from nerve endings, (*e*) blocking its synthesis, or (*f*) preventing its physiological release. So specific are some drugs in their action that they literally "map out" the peripheral nervous system: muscarine mimics faithfully the action of acetylcholine at parasympathetic sites; and curare differentially blocks the action of acetylcholine on myoneural junctions.

In the face of overwhelming evidence that drugs at the peripheral nervous system act by modifying synaptic transmission, many investigators are still loathe to assume that psychotherapeutic agents might act centrally in a similar way; instead, numerous workers still entertain the possibility that drugs influence behavior by affecting processes such as oxidative phosphorylation or carbohydrate metabolism. But it does not seem logical that drugs would exert unique effects on behavior by influencing processes so universal in nature that they occur even in plants; it seems more realistic to believe that these drugs affect the unique biochemical processes involved in synaptic transmission.

Among the trials that beset the study of drug actions in the central nervous system is the difficulty of isolating functional units. The central nervous system is a mass of intercommunicating neurons; one nerve cell affects innumerable others, and even large functional units influence one another. This makes it almost impossible to assign a drug effect, electrical or biochemical, to a particular brain site; depression induced in one part of the brain might reflect a direct action of the drug, or an indirect action, owing to loss of normal stimulating inflow. Another stumbling block existed as long as only one neurohormone, acetylcholine, was defined in the central nervous system. The discoveries in recent years of other potential neurohormones in

[1] The survey of the literature pertaining to this review was concluded in September, 1960.

brain—norepinephrine, dopamine, serotonin, γ-aminobutyric acid, and the polypeptide substance P—makes it possible to assume that drugs might interfere with the synthesis, storage, release, or destruction of any of these substances at central synapses.

By analogy with the peripheral nervous system, a drug might act so specifically with certain central synapses that it could serve to map out functional patterns of neurons in the central nervous system, and even to identify the distinctive neurohormones controlling their activity. The more specific the interaction between drug and synapse, the more discriminating will be the action of the drug. As a working hypothesis, it might be assumed that two compounds with an identical pharmacological profile might have a similar clinical application and influence the same functional unit. Unfortunately, this approach to the study of drug action is usually handicapped by the lack of pertinent pharmacological data on new drugs. Many studies describe the effects on one component of animal behavior, psychological adaptation, investigated by means of conditioned reflexes. Since behavior is the total integrated reaction to environmental change, the drugs ideally also should be studied for their effects on a variety of behavioral modalities: physiological, biochemical, electrophysiological, autonomic, and endocrine responses. Incidentally, some of these modalities might suggest simpler, more precise ways of screening drugs than those presently used. Only by observing the influence of drugs on the manifold facets of behavior will a deeper insight be gained into the neural pathways on which drugs act. The psychiatrist recognizes that abnormal autonomic activity might be an important aspect of distorted behavioral patterns; the pharmacologist should be equally aware that a drug-induced change in autonomic activity might play an important part in the action of the drug on emotion.

TRANQUILIZING AGENTS

With the advent of chlorpromazine and reserpine, the word "tranquilizer" was coined to describe drugs which evoke sedation without producing anesthesia. The development of compounds that elicit quiescence but with pharmacologic actions otherwise quite different from those of reserpine and chlorpromazine, has resulted in a heterogeneous assortment of drugs under the term, tranquilizer. The continued use of this term should serve as a constant reminder of our ignorance concerning their mode of action.

The unique properties of chlorpromazine and reserpine have been important not only in psychiatric therapy but in studies of biochemical and physiological processes in the central nervous system. But these drugs have not contributed to the total understanding of the schizophrenic process since they do not affect the fundamental pathology of the disease. Regardless of the psychiatric classification, they ameliorate only symptoms such as hallucinations, overactivity, aggressive behavior, anxiety, and tension, and thus enable the patient to cope with his psychosis. In this respect, they are in no way different from antihypertensive drugs now available which usually act

by preventing the action or release of norepinephrine but do not affect the disease process nor provide a clue concerning its etiology.

Chlorpromazine and Other Depressants of Central Sympathetic Tone (Central Adrenergic Blocking Agents)

Pharmacological aspects of chlorpromazine.—Many studies suggest that chlorpromazine is an adrenergic blocking agent which selectively antagonizes the action of norepinephrine in the central nervous system (1). In animals, the drug evokes a multifaceted syndrome whose salient features are tranquilization, depression of conditioned reflexes, decreased motor activity, lowered reactivity to external stimuli, depressed respiration, hypothermia, and a generalized lowering of sympathetic tone (2). All of these responses seem unrelated to each other until it is seen that dihydroxyphenylalanine which, unlike norepinephrine, readily enters brain to form dopamine (the precursor of norepinephrine) and some norepinephrine produces effects diametrically opposite to those of chlorpromazine: alertness, increased motor activity, enhanced reactivity to external stimuli, activated respiration, hyperthermia, and a generalized rise in sympathetic tone (3). Congeners of norepinephrine including ephedrine, deoxyephedrine, amphetamine, β-tetrahydronaphthylamine, methylphenidate and LSD-25,[2] which readily enter brain, elicit similar responses. Some of these drugs act on both the peripheral and central adrenergic systems; others like LSD-25 and methylphenidate exert mainly a central action. Not only do these drugs produce effects opposite to those of chlorpromazine, but they also counteract the effects of the tranquilizing agent presumably by competing for the same receptor sites.

The lowering of sympathetic tone by chlorpromazine may be attributed in part to mild peripheral adrenergic blockade but mainly to blockade of central sympathetic activity. Direct evidence that chlorpromazine suppresses central sympathetic activity is seen on its injection into the carotid artery or into the cerebral ventricles of monkeys, dogs, and cats; doses too small to be effective systemically produce sedation, relaxation of nictitating membrane, miosis, hypotension, and inhibition of the carotid sinus reflex (4, 5). The peripheral sympatholytic effects of chlorpromazine are slight; the drug blocks and even reverses the pressor effects of administered epinephrine, but not of administered norepinephrine (6, 7).

Absorption, distribution, metabolic fate and excretion of chlorpromazine.—Chlorpromazine is absorbed rapidly and readily enters the brain. Unlike reserpine, the drug acts quickly and exerts a sedative effect only while detectable in the central nervous system. Although chlorpromazine is very highly bound onto constituents of various tissues including brain, it does not accumulate in the body because of its relatively rapid metabolism by liver

[2] Since LSD-25 is an indole, it was formerly considered to act as an antiserotonin agent. Chemically it is also a phenylethylamine and its central effects are similar to those of other centrally acting norepinephrine congeners.

enzymes (8). Virtually all of the administered chlorpromazine is metabolized in the body. One of the metabolites, the sulfoxide of chlorpromazine, exerts mild chlorpromazinelike effects (9). Other metabolites, presumably hydroxylated and demethylated derivatives, have not yet been studied pharmacologically.

The individual variation in response to chlorpromazine, as well as to other psychotherapeutic agents, has posed a provoking problem to the investigator. The fact that drugs are inactivated at rates that may vary among different individuals by 500 per cent or more is seldom considered (10). Despite a decade of interdisciplinary research with chlorpromazine, the extent of individual variability in the metabolic degradation of this drug has not been investigated. It seems more practical, if less sophisticated, to relate variability in response with concrete factors such as differences in drug metabolism before trying to relate this variability with abstract factors like differences in personality structure. Moreover, the interpretation of carefully planned, double-blind studies, especially those with fixed dosage schedules, might be considerably different if it were known that the rates of chlorpromazine inactivation had varied by several hundred per cent among different individuals. Also lacking are data relating plasma levels with therapeutic efficacy or with occurrence of extrapyramidal side effects. This information might be useful in establishing dosage regimens, which by minimizing oscillations in drug plasma levels could result in greater effectiveness and fewer side effects.

Clinical aspects.—Numerous, well-controlled studies provide convincing evidence that chlorpromazine exerts therapeutic effects in psychoses and neuroses with anxiety. These and other clinical indications for the drug such as relief of nausea and vomiting and symptoms of acute alcoholism were discussed in the *Annual Review of Medicine* of 1958.

Recent studies on the mechanism of the antiemetic effects of chlorpromazine show that the drug blocks apomorphine and hydergine emesis. These results suggest that the drug depresses the chemoreceptor trigger zone in the area postrema (11).

Chlorpromazine produces undesirable effects of several types. Jaundice, allergic reactions, agranulocytosis, and convulsive seizures which require stopping the drug occur rarely and are usually reversible. Other effects like tachycardia, drowsiness, lethargy, fatigue, hypotension, and dizziness may require adjustment of dosage. One of the more perplexing effects of chlorpromazine is the occurrence of a Parkinson-like syndrome that usually disappears after stopping or reducing the drug, or which can be controlled by an antiparkinsonian drug. Some investigators feel that doses of chlorpromazine that elicit psychiatric improvement should not be lowered even though they produce extrapyramidal signs. But there is no experimental evidence to exclude the possibility that pushing chlorpromazine to the point of persistent parkinsonism might produce irreversible brain damage, despite masking of symptoms by means of an antiparkinsonian drug. In fact, there is

some indication in man that chlorpromazine can produce chromatolytic changes in neurons of basal ganglia and other brain structures (12).

The mechanism of the extrapyramidal effects of chlorpromazine is unclear. Electrophysiological studies demonstrate that chlorpromazine increases the rate of neuronal discharge in the midbrain reticular formation in response to paired stimulation of sciatic nerve and nuclei in the corpus striatum (13). This important structure of the extrapyramidal system contains very large amounts of dopamine (14). The possibility has been considered that dopamine in the corpus striatum has an important physiological role (15, 16) and that the extrapyramidal actions of chlorpromazine might be attributed to blocking of the amine. Favoring this view is the finding that reserpine, which depletes the dopamine in the corpus striatum, also elicits extrapyramidal signs. Moreover, both reserpine and chlorpromazine are used to control signs of Huntington's chorea (17, 18), certain manifestations of which are opposite to those of parkinsonism.

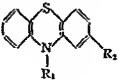
A number of reports indicate that chlorpromazine affects endocrine functions (1, 19, 20). It produces pseudogalactorrhea, can delay ovulation, and cause menstrual irregularity. Despite the fact that chlorpromazine lowers hypothalamic activity, it actually stimulates the pituitary-adrenal system. In rats, the same doses that elicit sedation also cause hypersecretion of ACTH, suggesting that its action on the pituitary is linked with the overall action on the hypothalamus (21).

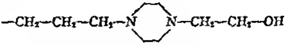
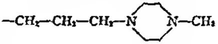
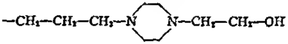
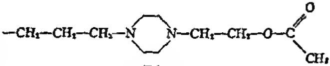
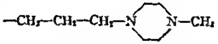
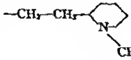
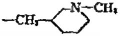
New phenothiazines.—Many congeners of chlorpromazine produce effects like those of chlorpromazine. Structurally they are all related by having a dialkylaminopropyl group attached to the ring nitrogen of phenothiazine. Deviation from the three carbon chain produces compounds that are not tranquilizers (22), for example Diparcol. Some of the new compounds are far more potent than chlorpromazine but it is difficult to conclude from available reports whether they represent a significant advance in psychiatric therapy, though they provide a greater clinical flexibility.

In many reports that claim a clear-cut advantage for one of the new drugs an arbitrary level of dosage is used which merely proves that drug A at one dosage is better than drug B at another. In many other investigations, controls are poor or lacking, criteria of improvement are vague, and details of experimental design and of results are inadequate. Lack of controls is especially pertinent in studies of psychoneuroses because of high rates of spontaneous remission and difficulty of assaying improvement. Information made available to the physician often stresses studies which show that a new drug benefits patients who did not respond to chlorpromazine. From data of this sort it might be concluded that the new drug is an improvement over chlorpromazine, but this view is difficult to defend. The particular individuals who failed to respond to chlorpromazine might have inactivated this drug with such facility that an effective plasma level could not be maintained, whereas the new drug was metabolized much more slowly. Again, the clinical status of the patient might have changed by the time the second

TABLE I

STRUCTURE OF PHENOTHIAZINE DERIVATIVES AND RANGE OF CLINICAL DOSES



Generic Name (Trade Name)	Range of Daily Oral Doses in mg.	R_1	R_2
Fluphenazine (Permitil, Prolixin)	0.5– 10		$-CF_3$
Trifluoperazine (Stelazine)	2– 30		$-CF_3$
[illegible]phenazine (Trilafon)	6– 24		$-Cl$
Thiopropazate (Dartal)	6– 30		$-Cl$
Prochlorperazine (Compazine)	15– 125		$-Cl$
Triflupromazine (Vesprin)	20– 150	$-CH_2-CH_2-CH_2-N(CH_3)_2$	$-CF_3$
Thioridazine (Mellaril)	30– 600		$-SCH_3$
Mepazine (Pacatal)	75– 400		$-H$
Promazine (Sparine)	25–1000	$-CH_2-CH_2-CH_2-N(CH_3)_2$	$-H$
Methoxypromazine (Tentone)	30–1500	$-CH_2-CH_2-CH_2-N(CH_3)_2$	$-OCH_3$
Chlorpromazine (Thorazine)	100–1000	$-CH_2-CH_2-CH_2-N(CH_3)_2$	$-Cl$

drug was tested. Rarely does one see the converse study—evaluation of chlorpromazine in individuals who failed to respond to one of the new drugs.

The more potent phenothiazines are reported to produce a lower incidence of lethargy, hypotension, and jaundice than does chlorpromazine. They occasionally cause leukopenia and agranulocytosis. Extrapyramidal symptoms are particularly marked with the more potent drugs (see Table I), but these can be controlled by antiparkinsonian agents.

Potential advantage of the new phenothiazines might be more apparent if information about their fate and physiological disposition in man were available. In studies of new drugs, the value of obviating individual variability in drug metabolism by comparing clinical effects in terms of plasma levels instead of dosage is still not appreciated despite past experiences with other therapeutic agents: mepacrine, thiobarbiturates, sulfonamides, and phenylbutazone, to name a few. In addition, rates of biotransformation would indicate whether or not doses of a particular drug can be widely spaced. All things being equal, it is easier to maintain therapeutic effectiveness with minimal toxic side effects if it is possible to avoid the oscillations of plasma level which, on the high side skirt toxicity and on the low side, therapeutic ineffectiveness. Data on an unusually high affinity for fat or tissue proteins might suggest dosage regimens that are more effective and minimize the occurrence of persistent toxic signs.

Reserpine and Other Compounds Which Increase Central Parasympathetic Tone (Serotonin-Depleting Agents)

Pharmacological aspects of reserpine.—Reserpine elicits a syndrome of effects comparable to that of chlorpromazine, including sedation, depression of conditioned reflexes, decreased motor activity, lowered reactivity to external stimuli, depressed respiration, and hypothermia (23).

Despite the similar clinical effects of reserpine and chlorpromazine, they act on different pathways in the central nervous system. For example, chlorpromazine depresses central sympathetic outflow, whereas reserpine stimulates central parasympathetic outflow (24). Miosis, lacrimation, salivation and perhaps bradycardia, increased gastrointestinal motility, and enhanced gastric secretion result from activation of central parasympathetic tone. The lowered sympathetic activity evoked by reserpine, formerly thought to be a central action, is now known to be mediated through the loss of norepinephrine from the peripheral stores (25). In accordance with this view, recent studies show that reserpine does not lower the frequency of impulses in preganglionic sympathetic fibers (26, 27).

A remarkable difference between reserpine and chlorpromazine is that the latter drug antagonizes the central excitation elicited by LSD-25 and amphetamine, whereas reserpine does not. Similarly, LSD-25-induced mental disturbances in man are ameliorated by chlorpromazine but aggravated by reserpine (28). Further evidence that the two drugs act on different systems is the facilitation by reserpine of electrically or chemically

induced convulsions in animals and man (29), whereas chlorpromazine does not exert this action (30).

An unusually interesting action of reserpine not shared by chlorpromazine is the enhancement of sensitivity to light. The blepharospasm in animals, and the prolonged miosis in animals and man, are probably caused by this action (24).

Biochemical aspects.—Although reserpine is metabolized extremely rapidly, its central actions persist for a considerable period of time, indicating that the drug does not act per se. When it was found that the drug produced a sustained depletion of brain serotonin stores, it was proposed that the change in brain serotonin and the central actions of the drug might be causally related (31). However, this interpretation seemed questionable when reserpine was shown also to lower the level of brain norepinephrine (32). A tool to solve this problem became available with the discovery of drugs which can selectively release norepinephrine (33). The evidence is now unequivocal that the central action of reserpine is related to its effects on brain serotonin rather than on brain norepinephrine. This has been most convincingly proved by means of α-methyl-m-tyrosine which, in mice, completely depletes norepinephrine with almost no change in brain serotonin; the animals treated with this synthetic amino acid evince no signs of sedation. However, if reserpine is now given to these animals, serotonin is lowered and sedation ensues (34). But despite the close association between the change in brain serotonin and the central actions of reserpine, a cause and effect relationship has not yet been established. However, it seems unlikely that the change in brain serotonin and the tranquilizing action are merely coincidental.

Reserpine exerts a direct action on the mechanism that maintains serotonin and norepinephrine within brain cells (30). Though reserpine impairs the capacity of cells to store amines, it does not affect their synthesis. Even after amine stores are depleted free amines are formed, but because of reserpine action they now freely diffuse from sites of synthesis. If a free amine forms at a slow rate, it might be metabolized by the enzyme monoamine oxidase so rapidly that it is unable to exert its physiological action. Reserpine may then be considered to have caused the exhaustion of the amine at the functional site. By contrast, if a free amine is made rapidly enough, its level at receptor sites may be sufficient to bring about persistent exaggeration of its normal physiological action. The possibility that reserpine actually increases the level of free serotonin at its functional sites is suggested by the extremely high rate at which this amine is synthesized in brain. It is thus possible that reserpine action in brain results from the continuous release of serotonin onto receptor sites (31), though this is still a working hypothesis.

Clinical aspects.—The clinical indications for the drug in mental illnesses are similar to those for chlorpromazine. Reserpine is less widely used than formerly because of its cumulative effects. The drug is also widely used in

hypertension; its therapeutic efficacy in this disease arises from the "chemical sympathectomy" produced by loss of peripheral stores of norepinephrine.

Some side effects of reserpine are related to changes in autonomic function; these include diarrhea, increased gastric secretion, postural hypotension, bradycardia, nasal congestion, and sensation of chilliness. Reserpine, like chlorpromazine, commonly causes extrapyramidal symptoms, especially a specific kind of motor restlessness called akathesia, which can be controlled with antiparkinsonian agents. In addition, the drug causes drowsiness, letbargy, fatigue, difficulty in swallowing and talking, and occasionally convulsions and allergic reactions.

Reserpine affects endocrine functions in various ways. In patients, it causes pseudogalactorrhea, menstrual irregularity, gynecomastia, reactivation of peptic ulcer, and facial edema (35). In view of the fact that reserpine reduces emotional activity it is surprising that in rats sedative doses of the drug produce an extraordinary hypersecretion of ACTH (36, 37). The increased secretion of ACTH and the sedation occur concurrently, suggesting that the action on the pituitary is part and parcel of its action on the central nervous system (38). If reserpine, as it is likely, also stimulates ACTH release in man, then we are in the embarrassing position of not knowing the biochemical and physiological consequences of this action after long-term treatment.

Absorption, distribution, and metabolic fate of reserpine.—It is difficult to maintain a constant clinical effect with reserpine. In fact, a severe and persistent depressive reaction can occur in patients receiving reserpine for treatment of hypertension. Two reasons make it difficult to control dosage: (*a*) On oral administration the drug is largely and perhaps variably broken down in the gut (39), presumably by the action of esterases; as a result, a single dose of the drug is much more effective intravenously than orally (40). (*b*) The drug is slow-acting and repeated doses exert cumulative effects over a period of weeks. A better reserpinelike compound might be one which is stable in the gut and does not exert cumulative effects.

Other Rauwolfia Alkaloids

The other natural alkaloids, deserpidine, raunescine, and rescinnamine, have also been used in treatment of mental disorders. They differ only quantitatively from reserpine in their clinical and pharmacological effects (23). There is no clear-cut evidence that these agents, in therapeutically equivalent doses, exert fewer side effects than reserpine.

Benzoquinolizines

Tetrabenazine (Nitoman) and other benzoquinolizines produce reserpine-like pharmacological effects in animals, including tranquilization, decreased motor activity, and a stimulation of central parasympathetic outflow (24, 41); these compounds, however, exert little effect on the peripheral sympathetic system (42, 43). Tetrabenazine causes a decrease in brain norepineph-

rine and serotonin, and only partially lowers peripheral amines. Unlike reserpine, tetrabenazine has a rapid onset of action. The effect of the drug on brain amines and its central effects are both of short duration, lasting only while the drug remains in the body (42, 44).

Insufficient reports are available to evaluate the drug clinically, but preliminary reports suggest that it is useful in treatment of hyperactive psychotic patients. The potential advantages of the drug lie in its rapidity of onset and lack of cumulative effects. The drug does not cause appreciable incidence of hypotension or diarrhea; like reserpine it evokes extrapyramidal signs (43).

Sites of Action of Reserpine and Chlorpromazine

Effects on the electrical activity of the nervous system.—Our knowledge of the functional and anatomical organization of the brain is too scanty to understand how drugs influence bioelectrical activity. Only in exceptional cases can we associate changes in electrical activity with changes in function; nevertheless, electrogenic responses in various brain regions might ultimately provide a crucial link in relating drug action to alterations in brain function. Changes in electrical activity have been of great importance in showing that reserpine and chlorpromazine act on different neural pathways.

Following the administration of chlorpromazine, sensory stimuli that formerly evoked behavioral and EEG arousal are no longer effective (45). However, unlike barbiturates, chlorpromazine does not depress the transmission of impulses in the brain stem reticular formation (46). Rather, it acts much more specifically to suppress the effects of sensory stimuli by interfering with the input from afferent collaterals onto this system. In contrast, norepinephrine and its congeners, amphetamine and LSD-25, produce an alerting of the EEG by sensitizing the animals to the influence of external stimuli (45). Experiments indicate that these phenylethylamines have little or no direct action on the brain stem reticular activating system but sensitize it to sensory influences, thus acting oppositely to chlorpromazine (47). These results support the view that chlorpromazine produces its tranquilizing action indirectly by blocking the activating effect of norepinephrine on the reticular formation. The animal remains conscious possibly because the activity of the reticular formation itself is not suppressed. According to this concept, norepinephrine controls the activating effect of afferent collaterals on the reticular formation; amphetamine and LSD-25 produce their central excitatory effects by mimicking the activating effect of norepinephrine on the reticular formation.

Since reserpine does not appreciably alter the response of the reticular activating system to sensory stimuli nor block the effects of norepinephrine or LSD-25 on this system, it may be inferred that reserpine and chlorpromazine produce similar behavioral effects by acting on different neuronal pathways. Reserpine, but not chlorpromazine, differentially affects the limbic system (48), an important part of the telencephalon, which includes the

amygdala, septum, hippocampus, pyriform cortex, and cingulate gyrus. The limbic system links the cortex anatomically and functionally with the hypothalamus, as shown by Papez (49). Reserpine initiates spontaneous electrical seizure patterns in the amygdala which may spread to the other limbic regions (50), but not to the neocortex; in addition, it lowers the threshold of excitability of the amygdala to electrical stimulation (51). The effect of reserpine on the amygdala may be an important clue to the mechanism by which this drug reduces the intensity of emotional behavior. In this regard it is pertinent that the amygdala is rich in serotonin but low in norepinephrine (52). Chlorpromazine does not activate the limbic system except in almost toxic doses (53); meprobamate and barbiturates decrease the excitability.

Unlike barbiturates, chlorpromazine and reserpine do not interfere with electrical activity in the classical sensory pathways. Failure of these tranquilizers to affect these pathways sharply differentiates their mode of action from that of hypnotics.

Effects of chlorpromazine and reserpine on central integrative systems.—Since the effects of tranquilizers cannot as yet be explained in terms of electrophysiological events, other experimental approaches are also needed to study their mode of action. One approach has been to compare alterations of total behavior induced by drugs with those produced by electrical stimulation through electrodes implanted in various brain regions. Hess (54) studied the means by which central autonomic pathways are integrated with other neural pathways to regulate behavioral mechanisms which can function without conscious control. He expounded the thesis that autonomic, skeletal muscle, and emotional behavior are coordinated by neuronal organizations in the subcortex made up of two functionally antagonistic systems, ergotropic and trophotropic. According to Hess, the ergotropic division integrates the central sympathetic system with somatomotor activities to produce behavior patterns which prepare the body for positive action (fight or flight reactions). The overall effects of ergotropic predominance—increased sympathetic activity, arousal, enhanced skeletal muscle tone and activity, and an activated emotional state—are startlingly similar to those of amphetamine and LSD-25, and conform to the view that these drugs stimulate while chlorpromazine blocks ergotropic activity. The trophotropic division integrates the central parasympathetic system with somatomotor activities to produce behavior patterns of a recuperative nature. The overall effects of trophotropic predominance—increased parasympathetic activity, drowsiness and sleep, decreased skeletal muscle tone and activity, and lowered responsiveness to external stimuli—are markedly similar to those of reserpine, and conform to the view that reserpine stimulates trophotropic activity.

On the basis of Hess's concepts, patients suffering from mental diseases characterized by symptoms of overactivity, aggression, and exaggerated emotional responses may be provided relief by drugs which can shift the

balance of subcortical activity toward normal, either by suppressing the ergotropic system (chlorpromazine) or by stimulating the trophotropic system (reserpine).

The view has been advanced that the ergotropic and the reticular activating systems might be intimately related and controlled by the action of norepinephrine. In fact, a detailed mapping of the brain amines shows that norepinephrine is localized largely in those regions under the influence of the reticular activating system (52). It has also been postulated that the trophotropic system is controlled by the action of serotonin (3). However, since serotonin is closely associated with norepinephrine in most areas controlled by the reticular activating system, the nature of the neural pathways associated with trophotropic activity remains undefined.

However, from recent studies showing that the brain stem reticular formation contains inhibitory fibers it has been postulated that inhibitory as well as excitatory pathways are dispersed diffusely throughout the reticular activating system (55). It is possible that serotonin is ossociated with these inhibitory fibers.

Central Muscle Relaxants with Psychosedative Properties

Meprobamate, a muscle relaxant drug with sedative action, was discussed in the *Annual Review of Medicine* of 1958. Methaminodiazepoxide (Librium), a compound with similar properties but an entirely different structure, is currently in style for the treatment of conditions dominated by psychological or muscular tension.

The pharmacological effects of methaminodiazepoxide and meprobamate differ from those of reserpine and chlorpromazine. Like barbiturates, they produce sedation and cause muscular relaxation by blocking polysynaptic pathways (56). The latter action, by relieving muscular tension, possibly accounts io part for their therapeutic effects. The drugs have no obvious influence on the hypothalamus for they do not affect autonomic functions. Finally, these drugs antagonize pentylenetetrazol (Metrazol) and electroshock seizures (56, 57).

Clinical aspects.—A number of reports, many of them based on uncontrolled studies, state that methaminodiazepoxide relieves anxiety and tension, and recommend its use in treatment of neuroses. Other papers indicate that it is useful in controlling graod mal epilepsy. As yet, available evidence does not permit the conclusion that this drug is more useful in treatment of neuroses thao a number of other mild sedatives. However, this does not mean that the drug may not ultimately prove to have some special merit, for evaluation of this sort of compound is complicated by the fact that many outpatients with neuroses improve on a placebo, and that barbiturates, if carefully used, are also effective.

Side effects, especially in larger doses, include drowsiness and impairment of thinking, ataxia, vertigo, confusinn, and an increase in appetite and weight. Occasionally, patients exhibit episodes of a ragelike reaction (58).

An important question is whether sedation and muscular relaxation, the two primary actions of the drug in animals, can account for its tranquilizing action in man. Based on experiments showing muscle relaxation in animals, the doses used in man seem small. But drugs are usually metabolized much more slowly in man than in animals, and comparative plasma levels would help answer this question.

ANTIDEPRESSANT DRUGS

There are four main types of antidepressant drugs: (*a*) phenylethylamine derivatives, (*b*) dimethylaminoethanol, (*c*) monoamine oxidase inhibitors, and (*d*) iminodibenzyl derivatives. The terms "thymoleptic" and "psychic energizer" have been applied to some of these drugs but these appellations seem even less helpful to the understanding of their action than the term "tranquilizer" for the sedative drugs.

Phenylethylamines

Central stimulants like amphetamine, and its milder congeners, pipradol (Meratran) and methylphenidate (Ritalin) are analogues of norepinephrine. They appear to stimulate adrenergic receptors in brain and induce effects opposite to those of chlorpromazine, including arousal, hyperactivity, and central sympathetic predominance. Electrophysiological studies suggest that these drugs produce their action by sensitizing the reticular activating system to sensory influences (59). The drugs are of some value in treatment of depressive neuroses and of behavioral problems in children, but have little use in more serious depressed states. Since they increase all central activity, whether healthy or psychotic in nature, the symptoms of subjects with delusions and anxiety may be heightened. The centrally acting phenylethlamines as a class show considerable selectivity in their action; they all depress appetite, but diethylpropion (Tenuate) and phenmetrazine (Preludin) are reported to do so over a wider range of dosage than amphetamine without increasing psychomotor activity (60). The more potent phenylethylamines generally evoke mental aberrations; LSD-25 provokes this effect at doses only little higher than those enhancing psychomotor activity, whereas very high doses of amphetamine and ephedrine are required.

Dimethylaminoethanol

Dimethylaminoethanol (deanol, Deaner) was tested on the premise that it would enter brain and be converted to free acetylcholine, but there is no evidence for this view. In animals, prolonged administration of deanol in large doses increases excitability and motor activity; in mice and rats the drug can elicit audiogenic seizures and in dogs spontaneous convulsive seizures (61).

The drug is reported to be useful in treatment of patients with simple depression and neuroses, and of children with behavior and learning problems. However, clinical evidence with this drug does not appear to be conclusive.

Monoamine Oxidase Inhibitors

Pharmacological and biochemical aspects of iproniazid.—The use of iproniazid in the treatment of mental depression came about from the observation that the drug produced euphoria and overactivity in tuberculous patients. An important lead in understanding the action of this hydrazine was provided when Zeller *et al.* (62) showed that it inhibits monoamine oxidase, an enzyme later found to be important in the metabolism of brain serotonin and norepinephrine. In certain animal species, iproniazid causes an increase in brain serotonin which is followed by an increase in brain norepinephrine. The drug induces excitation, increased locomotor activity, and mydriasis, which are temporally related to the rise in brain norepinephrine rather than the rise in brain serotonin. Further evidence that the central effects are not related to the rise in brain serotonin is the failure of iproniazid to cause excitation in dogs and cats; in these animals the drug elevates brain serotonin but not brain norepinephrine (63).

Iproniazid exerts anticonvulsant effects against electroshock and pentyleoetetrazol (64). In rats aod mice the rise in convulsive threshold coincides with the rise in brain amines. In contrast, reserpine, which depletes brain amines, lowers the convulsive threshold (29, 64).

Clinicol ospects of iproniazid.—Many reports (65, 66) indicate that iproniazid can relieve depression in some patients with involutional, eodogenous, or reactive psychoses; in other patients the drug is ineffective. The drug is often substituted for electroshock therapy, but in view of its slow onset of action, shock therapy is often preferred if there is risk of suicide.

The glow attendant upon the introduction of iproniazid has been dimmed by the occurrence of toxic effects. Postural hypotension, a frequent side effect, is a potential danger, especially in old patients who may suffer damage from falls. In certain patients the drug can convert a depressive reaction into one of toxic psychokinetic stimulatioo. A more serious side effect is occasional liver necrosis of a particularly toxic type, pathologically similar to viral hepatitis (67, 68). Because of this effect on the liver, the indiscriminate use of iprooiazid should be avoided.

New monoamine oxidase inhibitors.—The hepatotoxic action of iproniazid has stimulated the developmeot of other hydrazines, four of which have been marketed: 1-phenyl-2-bydraziooproproaoe (JB 516, Catron), phenylzine (Nardil), nialamide (Niamid), and isocarboxazid (Marplan). These compounds also raise brain levels of serotonin and norepinephrine and evoke a central excitation which coiocides with tbe rise in norepioepbrine. Two of the drugs, JB 516 and pbenylzine, also exert an amphetaminelike stimulatory action that appears sbortly after administration but is short-lived. In clinical doses, it is doubtful whether this action is important.

The drugs differ markedly in their potency, but, in general, the least toxic are also tbe least active. Clinical experieoce with the new compounds is still too limited to equate effectiveness with safety. They all produce orthostatic hypotension, the extent of which appears related to the degree of mono-

amine oxidase inhibition (69). The hypotension is suggestive of altered sympathetic function but the mechanism is unknown; nevertheless, the drugs are proving to be useful in the management of hypertension as well as in the treatment of angina pectoris.

Some of the newer hydrazines can produce liver and brain damage. Catron, the most effective of the drugs in the treatment of depression and hypertension, occasionally produces liver necrosis similar to that reported with iproniazid. In some patients, it has produced a reversible loss of red and green color discrimination, sometimes associated with diminished visual acuity and optic neuritis (70). Long-term administration of small doses of Catron (1 mg./kg.) and a closely allied congener, phenylisobutyl hydrazine, can produce lesions in the inferior olivary nucleus or in the pyriform cortex of dogs after 100 days of treatment (71). Yet, in rats and rabbits, this brain damage is not observed except after very large doses. This example of a delayed toxicity highlights the difficulty of setting up standards for toxicological evaluation of drugs that may have to be given for months or years. Because of its toxicity, Catron has been withdrawn from the market. Recently, another hydrazine monoamine oxidase inhibitor (serine-N-2-isopropylhydrazide) was withdrawn from clinical trial because it produced liver damage.

Monoamine oxidase inhibitors can modify the effects of other centrally acting drugs. By depressing the activity of the liver enzymes that inactivate barbiturates, they prolong the duration of action of these drugs. This potential hazard is probably not of clinical concern, for animal studies show that the effect on liver enzymes lasts only a short time after single doses of the inhibitors (72). The action of monoamine oxidase inhibitors can be aggravated by the stimulant effects of caffeine, so that patients on a monoamine oxidase inhibitor may become hyperexcitable after drinking coffee (73).

Since the inhibitors cause a marked rise in the levels of brain serotonin and norepinephrine, bizarre results might be expected if another drug is given which also acts at amine sites. For example, the activity of phenothiazines has been reported to be markedly increased after pretreatment of a patient with a monoamine oxidase inhibitor (73). Moreover, reserpine given to animals pretreated with a monoamine oxidase inhibitor can produce profound excitation rather than sedation (74). Perhaps only the fact that relatively small doses of reserpine are given to man has prevented catastrophic effects with this combination of drugs. Finally, as discussed later, imipramine has caused alarming symptoms and even death in subjects pretreated with a monoamine oxidase inhibitor (73). The fact that the effects of some drug combinations were first noted in the clinic underscores the importance of having more than routine animal data available before patients are subjected to a new psychotherapeutic agent.

Because of the potential toxicity of hydrazines, non-hydrazine monoamine oxidase inhibitors are being developed. Several, including N-benzyl-N-methyl-2-propanylamine (A 19120) and tranylcypromine (Parnate), are

being used clinically but at this writing their usefulness cannot be evaluated.

Absorption, distribution, and fate of monoamine oxidase inhibitors.—Like reserpine, monoamine oxidase inhibitors are "hit and run" drugs; inhibition of monoamine oxidase, rise in brain amines and pharmacological effects persist long after cessation of dosage despite the relatively rapid biotransformation of the drugs (75). Iproniazid is readily absorbed and quickly enters brain and other tissues. Although the drug is metabolized in a relatively few hours, monoamine oxidase blockade persists for a considerable time because of "irreversible" inactivation of reactive sites on monoamine oxidase. Since the drugs act "irreversibly" the effects of small daily doses are cumulative.

The peculiar mode of action of monoamine oxidase inhibitors poses the problem of dosage regulation, since the effects of these inherently dangerous drugs are related not to plasma levels, but to the extent of monoamine oxidase blockade. It is difficult to decide if a poor clinical response is caused by underdosage, or if side effects arise from overdosage. A simple test—the increase in urinary tryptamine or tyramine—makes it practicable and perhaps advisable to determine the degree of monoamine oxidase inhibition *in vivo* (76). These amines are ordinarily oxidized *in vivo* by the action of monoamine oxidase but after adequate blockade of the enzyme, there is a pronounced increase in their urinary excretion. This test might be particularly valuable in avoiding toxic effects in susceptible individuals. In addition, it might obviate the lag in onset of drug action by allowing the safe administration of loading doses. Last but not least, the test would be helpful in weeding out placebo effects in the screening of drugs.

Iminodibenzyl Derivatives

The discovery of imipramine (Tofranil) as an antidepressant shows the inherent danger of an inflexibly structured plan in choosing drugs for clinical trial. Recognition of imipramine as an antidepressant came about from the astuteness of Kuhn (77), who was testing the drug for a potential tranquilizing effect. Imipramine is structurally related to promazine (see Table I) except that the ring S is replaced by CH_2-CH_2 moiety.

Imipramine is not a monoamine oxidase inhibitor nor does it cause a rise in brain amines (78). The drug does not elicit excitation in animals; rather, its pharmacologic profile is that of a weak chlorpromazinelike compound, including slight drowsiness and the potentiation of barbiturate action (79). Thus, none of the screening procedures currently used would suggest this compound to be an antidepressant drug.

The antidepressant action of imipramine becomes dramatically evident in studies which show that in rats pretreated with the drug the following effects of reserpine are prevented: depression, miosis, diarrhea, ptosis, hypothermia, potentiation of hypnotics, and increased activity of the central parasympathetic system (80, 81). The action of chlorpromazine, however, is not prevented by the drug. The view has been suggested that imipramine might counteract reserpine by antagonizing the effects of free serotonin (80).

It is possible that the inhibition of reserpine action by imipramine might bear on its antidepressant action in man.

Absorption, distribution and fate.—On parenteral administration, imipramine is rapidly distributed throughout all tissues (82). The drug is highly localized in brain and other tissues; despite the high tissue affinity the drug is almost completely metabolized in rabbits within 6 to 8 hr. The antireserpine effect disappears in a few hours, showing that the drug is not a "hit and run" type like the monoamine oxidase inhibitors. After parenteral administration in man, the drug is rapidly absorbed but can be detected in plasma for only a short time because of its high affinity for various tissues. Plasma level curves indicate that there is considerable variability in different persons in the rate of metabolism of imipramine (83). Little of the drug is excreted unchanged and various hydroxylated and demethylated derivatives have been isolated from the urine and identified (82). The pharmacological and clinical activity of these products has not yet been reported.

In depressed subjects the effects of the drug are usually delayed for a period of three days to two weeks after starting treatment. It is possible that this delay results from cumulation of the drug or from an active metabolite.

Clinical aspects.—Investigators generally agree that imipramine can exert striking effects in endogenous and involutional depressions, and in selected cases of reactive depression. The drug is said to provide an effective alternative to electroshock therapy.

Unlike monoamine oxidase inhibitors, imipramine does not usually cause overactivity or euphoria, though in some patients it evokes tension and agitation. Side effects, usually transient and atropinelike in nature, include dryness of mouth, difficulty of eye accommodation, palpitation, sweating, constipation, and occasional episodes of manic excitement in patients with manic-depressive psychoses. Rarely, liver dysfunction and jaundice have occurred, which disappear when the drug is stopped.

Disastrous effects have occurred from combining a monoamine oxidase inhibitor and imipramine (84, 85). The patients who had been treated for some time with a monoamine oxidase inhibitor and then given imipramine developed bizarre motor signs, circulatory collapse, and hyperpyrexia. Somewhat similar signs occur in animals given this combination of drugs. It would seem to be inadvisable to treat a patient with imipramine too soon after giving a monoamine oxidase inhibitor.

CONCLUSION

Until recently the drugs used in clinical psychiatry appeared to act on the symptoms of mental disease by an extension of their pharmacologic effects in the normal organism. For example, chlorpromazine was first used in treatment of psychoses because of its selective sedative action in normal animals and man. With the discovery of the antidepressant affects of imipramine, there now comes the realization that some drugs may show a therapeutic action only on the abnormal mental state. This should be no great surprise

since many of our most useful drugs for the treatment of non-mental diseases show their characteristic therapeutic effect only in abnormal states: analgesics, antirheumatic agents, antiarrhythmic agents, drugs for heart failure, to mention a few.

The fact that imipramine elicits an antidepressant action in depressed patients but not in normal subjects may well result in a new philosophy in the search for psychotherapeutic agents in the hope of finding other drugs that affect only the abnormal central nervous system. The pharmacologist is now faced with the challenging problem of how to set up abnormal states in animals. Perhaps this might be achieved by the use of drugs that provoke abnormal central nervous system activity. This approach might prove to be a welcome respite from the generally used psychological screening procedures, which may have become too frozen in concept. These procedures, which successfully ignore the existence of neural pathways, were originally set up because they proved to be sensitive to chlorpromazinelike agents. The continued use of these methods is unlikely to uncover drugs with a different sort of activity.

LITERATURE CITED

1. Killam, E. K., "Psychopharmacology," 21–28, *Natl. Acad. Sci.-Natl. Research Council Publ.* (1959)
2. Courvoisier, S., Fournel, J., Ducrot, R., Kolsky, M., and Koetschet, P., *Arch. intern. pharmacodynamie*, 92, 305–61 (1953)
3. Brodie, B. B., Spector, S., and Shore, P. A., *Pharmacol. Revs.*, 11, 548–64 (1959)
4. Dasgupta, S. R., and Werner, G., *Brit. J. Pharmacol.*, 9, 389–92 (1954)
5. Spector, S., Bogdanski, D. F., and Brodie, B. B., *Federation Proc.*, 16, 337 (1957)
6. Journdan, F., Duchene-Marullaz, P., and Boissier, P., *Arch. intern. pharmacodynamie*, 101, 253–78 (1955)
7. Martin, W. R., Riehl, I. L., and Unna, K. R., *J. Pharmacol. Exptl. Therap.*, 130, 37–45 (1960)
8. Salzman, N. P., and Brodie, B. B., *J. Pharmacol. Exptl. Therap.*, 118, 46–54 (1960)
9. Moran, N. C., and Butler, W. M., Jr., *J. Pharmacol. Exptl. Therap.*, 118, 328–37 (1956)
10. Brodie, B. B., Maickel, R. P., and Jondorf, W. R., *Federation Proc.*, 17, 1163–74 (1958)
11. Glaviano, V. V., and Wang, S. C., *J. Pharmacol. Exptl. Therap*, 114, 359–66 (1955)
12. Roizin, L., True, C., and Knight, M., *The Effect of Pharmacologic Agents on the Nervous System*, 285–324 (Williams & Wilkins Co., Baltimore, Md., 1959)
13. Adey, W. R., Buchwald, N. A., and Lindsley, D. F., *Electroencephalog. and Clin. Neurophysiol.*, 12, 21–40 (1960)
14. Bertler, Å., and Rosengren, E., *Acta Physiol. Scand.*, 47, 350–61 (1959)
15. Carlsson, A., *Pharmacol. Revs.*, 11, 490–93 (1959)
16. Barbeau, A., *Neurology*, 10, 446–51 (1960)
17. Kempinsky, W. H., Boniface, W. R., Morgan, P. P., and Busch, A. K., *Neurology*, 10, 38–42 (1960)
18. Walther-Buel, H., *Encéphale*, 45, 771–75 (1956)
19. Woodbury, D. M., *Pharmacol. Revs.*, 10, 275–357 (1958)
20. Moretti, O., *Semana méd.* (Buenos Aires), 116, 954–55 (1960)
21. Maickel, R. P., and Brodie, B. B., *Federation Proc.*, 19, 267 (1960)
22. Himwich, H. E., *Science*, 127, 59–72 (1958)
23. Bein, H. J., *Pharmacol. Revs.*, 8, 435–83 (1956)
24. Bogdanski, D. F., Sulser, F., and Brodie, B. B., *J. Pharmacol. Exptl. Therap.* (In press)
25. Carlsson, A., Rosengren, E., Bertler, Å., and Nilsson, J., *Psychotropic Drugs*, 363–72 (Elsevier Publ. Co., Amsterdam, 1957)
26. Dontas, H. S., *J. Pharmacol. Exptl. Therap.*, 121, 1–7 (1957)

27. Iggo, A., and Vogt, M., *J. Physiol.*, 150, 114–33 (1960)
28. Himwich, H. E., *Neuropsychopharmacology*, 129–33 (Elsevier Publ. Co., Amsterdam, 1959)
29. Chen, G., Ensor, C. R., and Bohner, B., *Proc. Soc. Exptl. Biol. Med.*, 86, 507–10 (1954)
30. Tripod, J., *Psychotropic Drugs*, 437–47 (Elsevier Publ. Co., Amsterdam, 1957)
31. Brodie, B. B., *5-Hydroxytryptamine*, 64–83 (Pergamon Press, Ltd., London, Eng., 1957)
32. Holzbauer, M., and Vogt, M., *J. Neurochem.*, 1, 8–9 (1956)
33. Brodie, B. B., Finger, K. F., Orlans, F. B., Quinn, G. P., and Sulser, F., *J. Pharmacol. Exptl. Therap.*, 129, 250–56 (1960)
34. Kuntzman, R. G., Gessa, G. L., Costa, E., and Brodie, B. B., *Federation Proc.*, 20 (In press, 1961)
35. Gaunt, R., Renzi, A. A. Antonchak, N., Miller, G. J., and Gilman, M., *Ann. N. Y. Acad. Sci.*, 59, 22–35 (1954)
36. Kitay, J. I., Holub, D. A., and Jailer, J. W., *Endocrinology*, 65, 548–54 (1959)
37. Saffran, M., and Vogt, M., *Brit. J. Pharmacol.*, 15, 165–69 (1960)
38. Brodie, B. B., Maickel, R. P., and Westermann, E. O., *Proc. Intern. Neurochem. Symposium, Varenna, Italy, 4th Meeting* (In press)
39. Numerof, O., Gordon, M., and Kelly, J. M., *J. Pharmacol. Exptl. Therap.*, 115, 427–31 (1955)
40. Hoobler, S. W., and Blaquier, P., *Hahnemann Symposium on Hypertensive Disease, 1st Meeting*, 384–91 (W. B. Saunders Co., Philadelphia, Pa., 1959)
41. Pletscher, A., Besendorf, H., and Bächtold, M. P., *Arch. Exptl. Pathol. and Pharmacol.*, 232, 499–506 (1958)
42. Quinn, G. P., Shore, P. A., and Brodie, B. B., *J. Pharmacol. Exptl. Therap.*, 127, 103–9 (1959)
43. Stockhawsen, F. G., *Medicina Experimental.*, 2, 157–61 (1960)
44. Schwartz, D. E., Pletscher, A., Gey, K. F., and Rieder, J., *Helv. Physiol. et Pharmacol. Acta*, 18, 10–16 (1960)
45. Bradley, P. B., *Neuropsychopharmacology*, 11–19 (Elsevier Publ. Co., Amsterdam, 1959)
46. Killam, K. F., and Killam, E. K., *Reticular Formation of the Brain*, 111–22 (Little, Brown & Company, Boston, Mass., 1958)
47. Rothballer, A. B., *Pharmacol. Revs.*, 11, 494–547 (1959)
48. Killam, E. K., Killam, K. F., and Shaw, T., *Ann. N. Y. Acad. Sci.*, 66, 784–805 (1957)
49. Papez, J. W., *Arch. Neurol. Psychiat.*, 38, 725–45 (1937)
50. Sigg, E. B., and Schneider, J. A., *Electroencephalog. and Clin. Neurophysiol.*, 9, 419–26 (1957)
51. Costa, E., Morpurgo, G., and Revzin, A. M., *Biol. Psychiat.* (In press, 1960)
52. Kuntzman, R., Shore, P. A., Bogdanski, D., and Brodie, B. B., *J. Neurochem.* (In press, 1960)
53. Preston, J. B., *J. Pharmacol. Exptl. Therap.*, 118, 100–15 (1956)
54. Hess, W. R., *Das Zwischenhirn, Syndrome, Lokalisationen Funktionen*, 2nd ed. (Benno Schwabe, Basle, Switzerland, 1954)
55. Batini, C., Moruzzi, G., Palestrini, M., Rossi, G. F., and Zanchetti, A., *Science*, 128, 30–32 (1958)
56. Randall, L. O., Shallek, W., Heise, G. A., Keith, E. F., and Bagdon, R. E., *J. Pharmacol. Exptl. Therap.*, 129, 163–71 (1960)
57. Roszkowski, A. P., *J. Pharmacol. Exptl. Therap.*, 129, 75–81 (1960)
58. Tobin, J. M., Bird, I. F., and Boyle, D. E., *Diseases of Nervous System*, 21, Suppl. No. 3, 11–19 (1960)
59. Himwich, H. E., Van Meter, W. G., Owens, H., *Biological Psychiatry*, 27–52 (Grune & Stratton, Inc., New York, N. Y., 1959)
60. Barnes, R. H., *J. Am. Med. Assoc.*, 166, 898–903 (1958)
61. Pfeiffer, C. C., *Intern. Rev. Neurobiol.*, 1, 196–241 (1959)
62. Zeller, E. A., Barsky, J., Fout, I. R., Kirscheimer, F. A., and Van Arden, L. L., *Experientia*, 8, 349–50 (1952)
63. Spector, S., Shore, P. A., and Brodie, B. B., *J. Pharmacol. Exptl. Therap.*, 128, 15–21 (1960)
64. Prockop, D. J., Shore, P. A., and Brodie, B. B., *Ann. N. Y. Acad. Sci.*, 80, 643–51 (1959)
65. Feldman, P. E., *Ann. N. Y. Acad Sci.*, 80, 712–25 (1959)
66. Bailey, S. d'A., Bucci, L., Gosline, E., Kline, N. S., Park, I. H., Rochlin, D., Saunders, J. C., and Vaisberg, M., *Ann. N. Y. Acad. Sci.*, 80, 652–68 (1959)

67. Katz, R., Klinger, J., Silva, L., Rodriguez, J., and Ducci, H., *Ann. N. Y. Acad. Sci.*, 80, 898–914 (1959)

68. Popper, H., *Ann. N. Y. Acad. Sci.*, 80, 929–38 (1959)

69. Sjoerdsma, A., Gillespie, L., and Udenfriend, S., *Ann. N. Y. Acad Sci.*, 80, 969–80 (1959)

70. Gillespie, L., Jr., Terry, L. L., and Sjoerdsma, A., *Am. Heart J.*, 58, 1–12 (1959)

71. Maling, H. M., Highman, B., and Spector, S., *Federation Proc.*, 20 (In press, 1961)

72. Laroche, M. J., and Brodie, B. B., *J. Pharmacol. Exptl. Therap.*, 130, 134–37 (1960)

73. Kline, N. S., *Bull. World Health Organization*, 21, 397–410 (1959)

74. Shore, P. A., and Brodie, B. B., *Proc. Soc. Exptl. Biol. Med.*, 94, 433–35 (1957)

75. Hess, S., Weissbach, H., Redfield, B. G., and Udenfriend, S., *J. Pharmacol. Exptl. Therap.*, 124, 189–93 (1958)

76. Sjoerdsma, A., Oates, J. A., Zaltzman, P., and Udenfriend, S., *J. Pharmacol. Exptl. Therap.*, 126, 217–22 (1959)

77. Kuhn, R., *Schweiz. med. Wochschr.*, 87, 1135–40 (1957)

78. Pletscher, A., and Gey, K. F., *Helv. Physiol. et Pharmacol. Acta*, 17, 635–39 (1959)

79. Domenjoz, R., and Theobald, W., *Arch. intern. pharmacodynamie*, 120, 450–80 (1959)

80. Sulser, F., and Watts, J., *Proc. Intern. Symposium on Techniques for Study of Psychotropic Drugs, Bologna, Italy* (In press)

81. Costa, E., *Intern. Rev. Neurobiol.*, 2, 175–227 (1960)

82. Herrmann, B., and Pulver, R., *Arch. intern. pharmacodynamie*, 126, 454–69 (1960)

83. Greengard, P., and Quinn, G. P. (Personal communication)

84. Davies, G. I., *Brit. Med. J.*, No. 5204, 1019 (1960)

85. Singh, H., *Am. J. Psychol.*, 117, 360 (1960)

RESPIRATORY PROBLEMS OF THE NEWBORN[1]

By C. D. Cook, M.D.[2]

Children's Hospital Medical Center, and Department of Pediatrics, Harvard Medical School, Boston, Massachusetts

Introduction

It has long been realized that the major adjustment of the fetus to extra-uterine life involves extensive physiologic changes in the lungs particularly and in the cardiovascular system as well. Attempts to investigate the rapid and vital changes in the lungs in the perinatal period date back at least to the latter part of the nineteenth century (64); however, the work of Barcroft (12) in animals first delineated many of the important areas needing investigation, and Dawes and co-workers (43, 44, 45) have continued animal research that has provided descriptions of normal fetal and neonatal pulmonary and cardiovascular adjustments. Stimulated by these provocative experiments in animals and provided with a variety of new biochemical and physiologic techniques, investigations have recently been focused on the human newborn infant. Naturally, investigators have been intrigued by the puzzles involved in normal respiratory adjustments, but the studies are particularly important because they are allowing an ever-increasing understanding of the respiratory abnormalities encountered in the newborn, abnormalities which represent a challenge to obstetricians, pediatricians, anesthesiologists, biochemists, pathologists, and physiologists because they account for approximately half of all deaths in the first week of life (5, 13, 23, 26, 82). Hence, recent advances in the field of respiratory physiology of the newborn as well as advances in various types of respiratory problems will be reviewed; some of the research has necessarily been done on animals and only tentative extrapolation to newborn infants is possible at the present time.

Anoxia

One of the unique features of the fetus and newborn infant is its ability to withstand degrees of anoxia that would be damaging or fatal to the more mature organism. On the clinical level, it has been recognized for many years that the human fetus could tolerate relatively long periods of total oxygen deprivation but only recently have the protective mechanisms begun to be understood.

Himwich and his co-workers (66) showed that the resistance to anoxia was greatly decreased by the injection of iodoacetic acid or fluoride, both

[1] The survey of the literature pertaining to this review was concluded in August, 1960.

[2] This review was supported in part by research grants (H-3659 and H-5339) from the National Heart Institute, and by a grant (E-1170) from the National Institutes of Health, U. S. Public Health Service.

substances which inhibit enzymes in the glycolytic cycle. Villee and his coworkers (115, 116) have compared the *in vitro* metabolism of fetal and adult tissues in O_2 and N_2. From their experiments it was concluded that the glycolytic enzymes of the fetal liver respond to anaerobiosis more effectively than do those of the adult liver. These authors also tested, on the tissue level, the generally accepted belief that anoxia produces irreparable tissue damage. Both the liver and cerebral cortex cells were metabolically quite active after an hour of complete anoxia but it was not shown whether or not they could initiate or transmit nerve impulses. Histologic comparison of the tissues after exposure to O_2 and N_2 showed little difference, although chemical analysis indicated that much more glycogen had been utilized under anaerobic conditions.

It has been suggested by Villee (116) that "the differences between young and term fetal tissues observed in the rat, human, and goat represent a physiologic adaptation to persistent tissue hypoxia in the later part of gestation—enzymatic adaptations which would provide more energy without utilizing oxygen." And he concludes that the resistance of the mature fetus and newborn to anoxia is the result of several or many alterations in metabolic patterns each of which would contribute a small amount of energy.

In line with the studies on tissues, which indicate that the metabolism of glycogen is at least an important factor in anaerobic survival of tissues, is the interesting work of Dawes and his colleagues (46). In intact animals, these workers have shown a close correlation between the carbohydrate (glycogen) content of the cardiac muscle and the anoxic survival of newborn animals. Maintenance of the circulation is obviously of vital importance in determining survival, and hence there may be a causal relationship between the carbohydrate content of the cardiac muscle and resistance to anoxia.

Based on these fundamental studies, and of even potentially greater direct importance to the clinician, is work by Dawes, Mott & Stafford (47) which provides preliminary data on the treatment of anoxia in experimental animals. In addition to utilization of glycogen stores, a serious acidosis is also part of the picture of asphyxia. These workers have shown that correction of the acidosis combined with provision of glucose significantly prolongs survival under anoxic conditions and aids recovery from anoxia. It appears that much of the beneficial effect results from improvement of the cardiovascular status. However, at the present time it is too early to say whether or not brain damage may be prevented by such therapy. Although there is as yet no direct evidence that the same sort of process obtains in the respiratory distress syndrome of newborn infants, it is interesting to speculate that the therapy suggested by Reardon and her associates (95, 96) and by Usher (113, 114) may aid in the same sort of way. These workers have claimed beneficial effects from the administration of glucose and bicarbonate, lactate, or saline solutions and this may similarly help to maintain the circulation in distressed infants.

Recent studies by Moore (89) have shown that hypoxia in newborn kit-

tant factor in the expansion of the fetal lung. Pattle (91) studied the stability of bubbles from the lungs of guinea pigs and found less stability in the bubbles from the least mature lungs. At that time he postulated that an increase in surface tension, possibly arising from a deficiency of some factor, might be responsible for the atelectasis of the respiratory distress syndrome (hyaline membrane disease) encountered in the human premature infant. Because it was possible to extract a substance or substances with unique surface tension properties from postmortem lung specimens, it was soon shown by Avery & Mead (10) that lung extracts from human fetuses below 1100 or 1200 gm., had much higher (4 to 5 times) surface tension than similar extracts from more mature fetuses. High surface tension might well explain instability of the lungs, difficulty in expansion, and atelectasis.

Thus, progress is being made in the description of fundamental tissue changes during maturation of the lungs. The factor, or rather factors, for undoubtedly there are many, which are necessary for normal development, for adequate expansion and function of the lung on the one hand, and necessary for the avoidance of abnormalities on the other, are gradually being delineated.

Mechanics

In recent years, there has been much interest in the forces involved in the first breath as well as subsequent respiration (77). Prior to respiration, the lungs are fluid-filled and the introduction of air requires overcoming large surface tension forces. Thus, studies (61) on lungs of stillborn infants indicate that opening pressures of 15 to 25 cm. of H_2O are usually necessary and observations by Karlberg (76) have shown opening pressures as high as 60 cm. H_2O in some normal newborn infants. Although Jäykka (72) has suggested that "alveolar capillary erection" plays an important part in initial expansion of the lungs, it would appear that his interpretation of his *in vitro* experimental data is not valid. In addition, more critical experimental work by Avery and her co-workers (9) and others (56), shows that only a small expansile force can possibly be expected from the increased pulmonary vascular flow accompanying birth. That the respiratory muscles, even of the premature infant, are usually capable of producing the high pressures necessary, was shown in 1941 by Smith & Chisholm (104). As the fluid in the lungs is rapidly removed, the compliance of the lung (ΔVolume/ΔPressure) is increased until at one to two hours it would appear that, when compared on the basis of lung volume or lung tissue weight, the compliance of the infant's lung is similar to that of the adult's (33).

The application of this information about initial lung expansion lies in the field of newborn resuscitation; obviously, pressures of 25 cm. H_2O or more will be necessary (see above) for the first inflation of the lungs themselves. But what about additional pressures to expand the chest wall? In adults being artificially respired, almost half the pressure is used to overcome the elastic resistance of the thoracic structures. However, at least in newborn

tant biochemical and physiologic information will be obtained with these techniques. Whether or not they will actually be practical for resuscitation of apneic newborn infants or those with transitory respiratory insufficiency remains to be seen.

Respiratory aids are also necessary for the infant with the hyaline membrane syndrome and recently suggested methods for such treatment are mentioned under management of that condition.

Three recent publications (1, 2, 105) have reviewed the etiology of apnea and hypopnea of the newborn and pointed out the presently accepted techniques for treatment. Particular emphasis is placed on the importance of preventing situations that involve depression of the infant's respiratory center. Furthermore, the dangers of unnecessary and unskilled manipulations have been stressed. On the other hand, positive pressure (up to 25 cm. H_2O) ventilation has been recommended when necessary, and tracheal intubation when indicated when trained personnel are available.

Histochemistry and Biophysics of the Lungs

Previous reports by Potter (92) have provided an important description of the anatomical development of the air passages and alveoli and the pulmonary circulation. She has shown that one of the critical factors in the ability of the fetus to tolerate extrauterine existence is the extension of alveolar capillaries and the thinning of the alveolar walls so that the distance from alveolar air to blood is short enough for adequate exchange of O_2 and CO_2. More recently, Loosli & Potter (86) have described the development of elastic fiber systems within the lungs. They suggest that the elastic fiber is the key to the determination of lung architecture. Their work indicates that the lung of the newborn is structurally not a miniature of the lung of an adult. For example, in the premature infant the respiratory spaces are channels with vascularized walls without, or with only rudimentary, alveoli. At full-term birth, alveoli are small and incompletely developed and the limited elastic fibers allow growth of alveolar ducts and sacs.

Descriptions of histochemical and biophysical changes during lung maturation have suggested other factors which may be equally important in terms of lung differentiation and function. Sorokin and his co-workers (107) have examined the histochemistry of the developing lungs of rats, guinea pigs, and pigs and have shown that mesenchymal glycogen is found mainly in developing lungs of species that attain, at a relatively late time, a certain degree of chemical and architectural perfection. This time is called the "critical period" and is 59 per cent of the gestational period in sheep, 67 per cent in the guinea pig, 78 per cent or 7 months in man, and 113 per cent in the rat. Obviously, with different species birth occurs at various points in the anatomical and histochemical development of the lung, but in all species studied expansion of the lungs at birth is not accompanied by significant tissue chemical change.

In 1947, Gruenwald (62) drew attention to surface tension as an impor-

tant factor in the expansion of the fetal lung. Pattle (91) studied the stability of bubbles from the lungs of guinea pigs and found less stability in the bubbles from the least mature lungs. At that time he postulated that an increase in surface tension, possibly arising from a deficiency of some factor, might be responsible for the atelectasis of the respiratory distress syndrome (hyaline membrane disease) encountered in the human premature infant. Because it was possible to extract a substance or substances with unique surface tension properties from postmortem lung specimens, it was soon shown by Avery & Mead (10) that lung extracts from human fetuses below 1100 or 1200 gm., had much higher (4 to 5 times) surface tension than similar extracts from more mature fetuses. High surface tension might well explain instability of the lungs, difficulty in expansion, and atelectasis.

Thus, progress is being made in the description of fundamental tissue changes during maturation of the lungs. The factor, or rather factors, for undoubtedly there are many, which are necessary for normal development, for adequate expansion and function of the lung on the one hand, and neces- sary for the avoidance of abnormalities on the other, are gradually being delineated.

Mechanics

In recent years, there has been much interest in the forces involved in the first breath as well as subsequent respiration (77). Prior to respiration, the lungs are fluid-filled and the introduction of air requires overcoming large surface tension forces. Thus, studies (61) on lungs of stillborn infants indicate that opening pressures of 15 to 25 cm. of H_2O are usually necessary and observations by Karlberg (76) have shown opening pressures as high as 60 cm. H_2O in some normal newborn infants. Although Jäykka (72) has suggested that "alveolar capillary erection" plays an important part in initial expansion of the lungs, it would appear that his interpretation of his *in vitro* experimental data is not valid. In addition, more critical experimental work by Avery and her co-workers (9) and others (56), shows that only a small expansile force can possibly be expected from the increased pulmonary vascular flow accompanying birth. That the respiratory muscles, even of the premature infant, are usually capable of producing the high pressures neces- sary, was shown in 1941 by Smith & Chisholm (104). As the fluid in the lungs is rapidly removed, the compliance of the lung (ΔVolume/ΔPressure) is increased until at one to two hours it would appear that, when compared on the basis of lung volume or lung tissue weight, the compliance of the infant's lung is similar to that of the adult's (33).

The application of this information about initial lung expansion lies in the field of newborn resuscitation; obviously, pressures of 25 cm. H_2O or more will be necessary (see above) for the first inflation of the lungs them- selves. But what about additional pressures to expand the chest wall? In adults being artificially respired, almost half the pressure is used to overcome the elastic resistance of the thoracic structures. However, at least in newborn

animals, the thorax, as might be expected, appears relatively very compliant. This has been shown well by Agostoni in puppies (4) and is confirmed by the work of Avery & Cook (11) in goats. Although the studies in humans have not been extended to the newborn, the trend toward a relatively more compliant thorax in younger children has been shown (35). Thus, the principal considerations in the artificial expansion of the lungs of the newborn infant are the lungs themselves, their surface tension, and elastic forces.

Techniques

There has been a great surge of interest in respiration and respiratory problems of the newborn infant and this has been facilitated by the availability of improved techniques. The development of cineradiography has allowed observation of the aeration of the lungs from the very first gasp (78) and swallow (21). Cardiac catheterization during the neonatal period (3, 84, 85, 98) has provided information concerning pulmonary circulatory adjustments both in the normal infant and in the infant with respiratory distress. Methods of microanalysis for blood gases have provided valuable data on the effectiveness of the lungs in performing their principal function of gas exchange under varying conditions (68, 96, 117). The ability to measure ventilation under relatively basal conditions with body plethysmographs perfected by Cross (39) and reverse plethysmographs (48), as well as with flow meters (88), has added to the knowledge of the newborn infant's respiratory adaptation to extrauterine life. Lung compliance and flow resistance have been studied, using intraesophageal catheters (32) and balloons (112) for the estimation of intrapleural pressure changes. These techniques have been applied by Karlberg and associates (79) to an examination of the forces involved in the first breath. Total chest and lung compliance of the newborn infant have been estimated by Weisser and his associates (118).

Static lung volumes have been measured by two different procedures. Berglund & Karlberg (17) have measured the functional residual capacity of both normal infants and those with respiratory distress, using a helium-dilution method, while Klaus and associates (81) and Auld and his co-workers (8) have measured functional residual capacity by the application of the Boyle's law technique suggested for adults by Dubois *et al.* (50). Gas exchange has also been measured (31, 41, 73, 108) and these studies should be greatly aided by the use of a small but low-resistance valve devised by Golinko and associates (59) and a tiny Rahn sampler designed by Nelson (90). Even lung diffusion has been studied by Stahlman & Meece (108, 109) who applied the steady-state carbon monoxide method devised by Filley (55) to the newborn infant.

All of these techniques, coupled with the histochemical and biophysical description of the lungs previously mentioned, are opening up new areas of investigation in the newborn infant, and allowing extension of knowledge to the human newborn organism where previously most of the information has had to be based solely on animal experimentation.

Control of Respiration

Indicative of the need for further investigation in the area of respiration of the newborn infant is the disagreement on the relative importance of various factors involved in the initiation of respiration. The work of Barcroft (12) and, more lately, Dawes (44), in animals (sheep and monkeys), has shown that tactile and thermal stimuli as well as severe anoxia all participate in initiating respiration under experimental conditions. James, Weisbrot and their associates (68, 117) have measured blood pH, CO_2, and O_2 in a large series of infants and have concluded that the high P_{CO_2} and low pH encountered in the blood of most newborns probably play major roles in the first gasp if anoxia is not severe. Their studies on asphyxiated infants indicated that, although the iofant might make a gasp when there was no measureable O_2 in the umbilical arterial blood, O_2 seemed essential for rhythmical activity of the respiratory center in the presence of severe respiratory and metabolic acidosis. They felt that "failure to breathe at birth is caused by either depressant drugs or the severity of the respiratory and metabolic acidosis, the result of prolonged asphyxia."

Reardon and her co-workers (96) agree that at birth there is a mild metabolic and respiratory acidosis which is possibly caused by the continued production of CO_2 at a time when the lung is non-functioning and the placental circulation is impaired. Their data show that within an hour after birth the respiratory acidosis has been corrected but the newborn infant apparently cannot compensate for the metabolic acidosis by hyperventilation. Later, between 4 and 24 hr. of age, more adequate ventilation apparently lowers the P_{CO_2}, sometimes to the point of respiratory alkalosis. The fact that after 4 hr. P_{CO_2} is decreased and P_{O_2} is not in the range expected to influence respiration, suggested "that plasma pH is the major chemical stimulus to respiration from 4 hours after birth through the first 3 days of life."

Still another concept concerning the control of respiration is that of Graham & Wilson (60) who conclude that O_2 lack is one of the most important factors in stimulating respiration in the newborn infant.

Whatever the relative importance of various factors in initiating and regulating respiration, it is apparent that the margin of safety in the first few hours and days is small; if there is any significant degree of pulmonary abnormality, acidosis will usually result.

Hyaline Membrane Syndrome

Of all the respiratory abnormalities encountered in the newborn period, the hyaline membrane syndrome, or the respiratory distress syndrome as it is sometimes called, has aroused the greatest interest because it is the single most frequent cause of death in newborn infants (5, 26). In an excellent report, James (69) has summarized much of the presently available clinical and research information. Although particular interest in this condition dates

back to the reports by Farber & Wilson in 1932 and 1933 (53, 54, 120), in the present review only the newer data will be mentioned.

Attempts to produce the pathological picture of hyaline membrane disease in the lungs of animals have not been successful. The membranes have been produced by exposure to high concentrations of O_2 for long periods of time (2 to 4 days) (16), and by the injection of plasma into the trachea (19), but atelectasis, which is so prominent a part of the naturally occurring condition, has not been consistently present. However, the report of Alvizouri (6) deserves mention because he has been able to produce hyaline membranes with injections of plasma and 0.22 per cent $CaCl_2$ directly into the trachea of rabbits. Apparently when this mixture comes in contact with the thromboplastin contained in the lung parenchyma, a clot of fibrin is formed. Although this experimental technique obviously does not simulate the steps involved in the naturally occurring disease, nevertheless, it may provide a reproducible method of studying the time-course of the resorption of the membranes and provide a preparation which could be used to test various therapeutic regimens. Much like the process in infants, the experimental membrane thus produced is well developed in 12 hr. and begins to recede toward the third or fourth day and has practically disappeared by the sixth.

The studies of Gitlin & Craig (58) in 1956 identified the hyaline material lining the alveolar ducts as fibrin, the source apparently being serum transudates into the air spaces. Their further work (38) showed in postmortem specimens that the membranes blocked the air passages. Mahaffey & Rossdale (87) have reported that a disease which simulates the hyaline membrane syndrome occurs in foals, particularly when the mare is attended. If further study confirms this, it will be of great interest (in spite of the fact that the horse is an expensive experimental animal) as it will be the first instance of this condition in animals.

It has already been mentioned that Avery & Mead (10) have shown a relationship between prematurity and the presence of high surface tension forces demonstrated by the lung extract studies. Furthermore, they have shown that infants dying of hyaline membrane disease, regardless of their gestational age (although most, of course, are premature infants), usually have lung extracts exhibiting higher surface tension forces than does the control material. They have postulated that this abnormality is at least one of the important causative factors in the pathogenesis of the atelectasis of the hyaline membrane syndrome. It has even seemed possible that the change in intrapulmonary pressure secondary to the high surface tension forces might in some way account for part of the transudation of plasma which must precede the precipitation of fibrin.

Other workers (101) have favored cardiovascular factors as most important in producing the clinical picture. Rudolph and his associates (98) have shown that once the clinical picture is present these infants have an abnormal left-to-right shunt through the ductus arteriosus which may

aggravate the pulmonary congestion. The question that arises, however, is whether the cardiovascular changes are the cause of the disease or merely the effect.

At least consistent with the physiologic studies of Rudolph are the findings of Burnard (27, 28). He has demonstrated that the heart size was larger in newborn infants with respiratory distress and decreased as symptoms improved. In addition, there was an associated crescendo systolic murmur that suggested a left-to-right shunt, presumably through the ductus arteriosus. A reduction in body temperature seemed to result in improvement of the dyspnea and disappearance of the murmur and, conversely, warming three infants shortly after birth brought about dyspnea as well as the murmur. Burnard suggests that the early roentgenographic appearance in the respiratory distress syndrome could represent pulmonary congestion and developing edema and the autopsy findings are at least consistent with vascular congestion. Thus, left-sided failure may, as a result of asphyxia in the perinatal period, lead to the symptoms of hyaline membrane disease. Unfortunately, treatment with digitalis has thus far produced only equivocal results so that this hypothesis needs further physiologic and therapeutic proof.

Lieberman & Kellogg (83) have found that there is a decrease in fibrinolytic activity in the lung tissues of infants dying with hyaline membrane disease and have added one more hypothesis concerning the pathogenesis of the hyaline membrane syndrome. These workers have suggested that in certain cases fibrin precipitates may not be lysed because of a deficiency of normal tissue fibrinolytic factors; if this were so, the hyaline membranes might be explained. On the other hand, the deficiency may equally well be secondary to the presence of excessive amounts of fibrin; this is suggested by other work (99) which has shown a decrease in serum fibrinolytic activity in infants dying of respiratory distress, but not in those who survive.

In an attempt to learn more about the pathogenesis of the hyaline membrane syndrome, Cohen, Weintraub & Lilienfeld (30) reviewed the obstetrical histories of 2001 premature and 24,108 full-term infants. They found that if there was no evidence of unusual uterine bleeding (as with placenta praevia, for example), then the incidences of the hyaline membranes at postmortem for infants delivered by cesarean section and those delivered vaginally were not significantly different. When there was evidence of intrauterine hemorrhage, the incidence of hyaline membranes was 14 to 16 times as great as when bleeding was not present. They postulated that the hyaline membrane syndrome might result from maternal bleeding that might, in turn, lead to placental dysfunction and acute hypoxia. These authors' data tend to confirm the earlier work of Strang, Anderson & Platt (111) who showed no relation between the hyaline membrane syndrome and uncomplicated cesarean section and concluded rather that fetal distress was one of the important pathogenetic factors.

Snyder (106) has stressed the importance of intrauterine hemorrhage

back to the reports by Farber & Wilson in 1932 and 1933 (53, 54, 120), in the present review only the newer data will be mentioned.

Attempts to produce the pathological picture of hyaline membrane disease in the lungs of animals have not been successful. The membranes have been produced by exposure to high concentrations of O_2 for long periods of time (2 to 4 days) (16), and by the injection of plasma into the trachea (19), but atelectasis, which is so prominent a part of the naturally occurring condition, has not been consistently present. However, the report of Alvizouri (6) deserves mention because he has been able to produce hyaline membranes with injections of plasma and 0.22 per cent $CaCl_2$ directly into the trachea of rabbits. Apparently when this mixture comes in contact with the thromboplastin contained in the lung parenchyma, a clot of fibrin is formed. Although this experimental technique obviously does not simulate the steps involved in the naturally occurring disease, nevertheless, it may provide a reproducible method of studying the time-course of the resorption of the membranes and provide a preparation which could be used to test various therapeutic regimens. Much like the process in infants, the experimental membrane thus produced is well developed in 12 hr. and begins to recede toward the third or fourth day and has practically disappeared by the sixth.

The studies of Gitlin & Craig (58) in 1956 identified the hyaline material lining the alveolar ducts as fibrin, the source apparently being serum transudates into the air spaces. Their further work (38) showed in postmortem specimens that the membranes blocked the air passages. Mahaffey & Rossdale (87) have reported that a disease which simulates the hyaline membrane syndrome occurs in foals, particularly when the mare is attended. If further study confirms this, it will be of great interest (in spite of the fact that the horse is an expensive experimental animal) as it will be the first instance of this condition in animals.

It has already been mentioned that Avery & Mead (10) have shown a relationship between prematurity and the presence of high surface tension forces demonstrated by the lung extract studies. Furthermore, they have shown that infants dying of hyaline membrane disease, regardless of their gestational age (although most, of course, are premature infants), usually have lung extracts exhibiting higher surface tension forces than does the control material. They have postulated that this abnormality is at least one of the important causative factors in the pathogenesis of the atelectasis of the hyaline membrane syndrome. It has even seemed possible that the change in intrapulmonary pressure secondary to the high surface tension forces might in some way account for part of the transudation of plasma which must precede the precipitation of fibrin.

Other workers (101) have favored cardiovascular factors as most important in producing the clinical picture. Rudolph and his associates (98) have shown that once the clinical picture is present these infants have an abnormal left-to-right shunt through the ductus arteriosus which may

Treatment of Hyaline Membrane Syndrome

A number of descriptions of the pulmonary and biochemical changes accompanying the hyaline membrane syndrome are available (32, 49, 69, 74, 110). The data indicating that the lungs are inelastic and that the infant has to work extraordinarily hard to breatbe, coupled with the clinical picture of severe respiratory distress and progressive cyanosis and finally respiratory failure, have led to attempts to aid these infants' respiration. Both Benson & Celander (15) and Bucci (25), among others, have attempted artificial tracheal insufflation to tide infants over the period when respiratory symptoms are most severe; the efficacy and practicality of such procedures still need further evaluation.

Approaching therapy from the point of the deranged blood chemistries [a respiratory and metabolic acidosis (71, 94) and a tendency toward a high serum potassium], Reardon and associates (95) and Usher (113, 114) have advocated the administration of saline or alkalinizing solutions containing glucose. Although the results of such attempts at therapy are difficult to evaluate, it is at least interesting that the regimens are similar to those found by Dawes, Mott & Stafford (47) to be most useful in the treatment of experimental asphyxia (see above).

Recently, a number of investigators have become interested in the use of low environmental O_2 tensions, and still others in the application of hypothermia to the prevention of neonatal respiratory distress and its treatment. Basing their regimens on the fact that P_{O_2} is low during fetal existence (and especially so in asphyxiated infants), Sjöstedt & Rooth (103) have suggested that exposure even to the O_2 tension of room air might in some way produce pulmonary vascular congestion. In experiments in newborn rats, these authors (97) showed that the lungs weighed more when the rats had been in air for 4 to 7 hr. than when they had breathed 15 per cent O_2 for the same period of time, and if the rats were in 50 per eent or 100 per cent O_2 the lungs were still heavier. They have tried a 15 per cent O_2 atmosphere as routine prophylactic management for a number of full-term and premature infants for periods ranging from a few hours to five weeks without apparent damage or risk. However, there is as yet no evidence that such a regimen has any value and it certainly should be tried with great caution in any newborn infants who already have an O_2 deficit since hypoxia in distressed newborn infants produces hypoventilation (74).

The use of low O_2 tensions received further attention when Cross and co-workers (42) showed that sueh treatment reduced O_2 consumption. However, as mentioned above, Hill (65) has shown that this effect is not found if the subject is maintained at the neutral environmental temperature. Thus, at present there is no evidence that hypoxia either for prophylaxis or treatment of distressed infants is useful and it should be considered an experimental procedure to be used only under the most carefully controlled conditions.

in the development of the hyaline membrane syndrome but has felt that actual aspiration of blood was the prime factor. Calkins & Miller (29) have described some of the clinical features as well as the correlation with the obstetrical history. The relative part played by these and other factors is at present unknown, but the known and questionable factors are summarized in Table I.

TABLE I*

INFORMATION CONCERNING HYALINE MEMBRANE DISEASE (34)

CERTAIN	PROBABLE	POSSIBLE
Clinical		
Not found in stillborn	Infants of diabetic mothers predisposed	Less common in India
More common the more premature the infant	Recovery occurs	Cesarean section may predispose to disease
Death may occur in 45 min. to several days	No residual lung or heart disease	
Sometimes occurs in full-term infants	Tends to occur in siblings	
No hydropic infants and only some in congestive failure get the disease		
X-ray may be initially clear, with progression to reticulogranular pattern		
Symptoms begin at or soon after birth		
Pathologic		
Atelectasis always present	Membrane is obstructive	Epithelial cell injury
Membranes located in proximal alveoli, overdistended alveolar ducts, and terminal bronchioles	Fibrinogen from circulation and thromboplastin from amniotic fluid to form fibrin	Lung weight greater than expected
Contain fibrin	May be ingested by macrophages	
Biochemical		
During illness, pH and P_{CO_2} tend to be low	Total body water increased	Serum potassium elevated
Lung tissue deficient in surface-active material	Hyperbilirubinemia present	
	Lung tissue deficient in profibrinolysin	
Physiologic		
Respiratory rate increased	Tidal volume normal or slightly decreased	End expiratory intrapleural pressure more negative
Compliance decreased	Functional residual volume decreased	
Dead space increased	Cardiomegaly	
Work of breathing increased	Systemic and pulmonary hypotension	
Aorta-to-pulmonary shunt through ductus arteriosus		

* Reproduced, with permission, from "Advances in Pediatrics," Volume XI, 1960, The Year Book Publishers, Inc.

approximately 2000 sera, and all of these additional patients were found to have chronic pulmonary disease. Recently, an infant with chronic respiratory distress dating back to $2\frac{1}{2}$ months of age was also found to have milk precipitins (36) and it appears that this test should be applied to all otherwise unexplained serious respiratory disease. It is certainly too early to know the results of treatment of these persons with milk-free diets but it is at least possible that some cases of chronic bronchiectasis and respiratory insufficiency may be prevented if properly diagnosed in early infancy.

Neonatal Pulmonary Infections

A number of workers (5, 23, 82) have pointed out that pneumonia is a frequent cause of respiratory distress in the newborn infant and, moreover, frequently complicates the hyaline membrane syndrome. The diagnosis is difficult but should always be suspected particularly when there has been premature rupture of the membranes. Blanc (18) and Benirschke (14) have stressed the value of histological examination of the placenta and membranes in providing information about the possibility of perinatal aspiration pneumonia. Eichenwald & Shinefield (52) have done much to clarify the means of spread of postnatal infections within a newborn nursery, and Eichenwald's demonstration of the symbiotic effect of viruses and bacteria (51) has been an important contribution.

Since the advent of effective drugs for the treatment of tuberculosis, a certain casualness has developed in the prevention of this condition. That this is indeed dangerous is shown by the report of Kendig (80) who found that of 41 infants born to mothers with supposedly inactive tuberculosis, 20 became infected with the disease. Even in the group of 16 infants who were separated at birth from their mothers for periods ranging from seven weeks to two years, eight developed tuberculous infections. Certainly, the use of BCG vaccine should be seriously considered for such high risk patients.

Summary

Within this large field with its necessarily divergent approaches to the biochemical, physiological, and clinical information concerning respiratory abnormalities of newborn infants, one of the most important unifying factors has been the sense of need. Thus, the recognition of the large number of potentially preventable deaths in this age group has led to an ever-increasing interest in fundamental research. It should be apparent from the foregoing review that considerable progress has been made and that techniques are available which may well allow solution of many of the problems.

LITERATURE CITED

1. "Resuscitation of the Newborn Infant," *Am. Acad. Pediat.*, Booklet (1958)
2. Committee Report, *Obstet. and Gynecol. N. Y.*, 8, 336–61 (1956) (Medical Society, County of New York)
3. Adams, F., and Lind, J., *J. Pediat.*, 19, 431 (1957)
4. Agostoni, E., *J. Appl. Physiol.*, 14, 909 (1959)
5. Ahvenainen, E. K., *J. Pediat.*, 55, 691 (1959)

Mild hypothermia has also been used (75) in an attempt to reduce metabolic requirements in the respiratory distress syndrome but no really adequate studies are available which allow evaluation of its efficiency. It would appear from the experimental work of Hill (65) (see above) that changes in the body temperature of normal newborn animals may at times increase O_2 consumption rather than reduce it. And this author's preliminary experiments in premature infants as well as those of Brück (24) suggest that infants react to a slightly lowered environmental temperature by increasing O_2 consumption just as do other mammals. The effect of hypothermia on the respiratory center as well as on total body metabolism of infants in respiratory distress remains the subject of future important investigation.

Another approach to therapy of the hyaline membrane syndrome has been suggested by the work of Craig, Fenton & Gitlin (38) and by Lieberman & Kellogg (83). It has seemed possible that the addition of fibrinolytic substances might aid in the resolution of the fibrin membranes. This occurred in tissue slices exposed to high concentrations of these substances for long periods of time. However, the work of Sherry and co-workers (102) as well as that of Samartzis *et al.* (99) has shown that the preparations currently available are relatively impotent.

The possibility that an antiatelectasis factor can be extracted from the lung tissue of animals is being explored by Bondurant (20) and this might prove to be useful in correcting the high surface tension present in the lungs of infants with the hyaline membrane syndrome. However, much work needs to be done before such therapy can even be tried, much less evaluated.

Other Respiratory Problems

Another type of respiratory disease in premature infants has been reported by Wilson & Mikity (121). The principal clinical features of this condition were that it occurred in prematures in the first month of life and had a gradual onset with hyperpnea and cyanosis. The chest was usually overexpanded and wheezing and coughing were present, but no fever. The chest x-ray presented a striking picture of coarse, reticular infiltration and cystlike appearance. There was a tendency to develop cor pulmonale, with cardiac and respiratory failure or superimposed infection as a cause of death. Although there is at present no known specific therapy, two of the five infants survived. Microscopic examination of lung tissue revealed alveolar septal thickening and fibrosis in three cases. There may be multiple etiological factors producing the picture described by these authors; certainly, further observations are needed in order to understand the nature of the process, which appears similar to the Hamman-Rich syndrome described in older persons, and its treatment.

Another interesting entity has been described by Heiner & Sears (63). These authors reported that three of 65 persons with chronic, serious, idiopathic bronchiectasis have shown, in their sera, precipitins against milk. An additional three persons with such precipitins were picked up by studying

J. H., Jr. *J. Clin. Invest.*, 35, 322 (1956)
51. Eichenwald, H. F., *J. Diseases Children*, 96, 438 (1958)
52. Eichenwald, H. F., and Shinefield, H. R., *J. Pediat.*, 56, 665 (1960)
53. Farber, S., and Wilson, J. L., *Arch. Pathol.*, 14, 437 (1932)
54. Farber, S., and Wilson, J. L., *Arch. Pathol.*, 14, 450 (1932)
55. Filley, G. F., MacIntosh, D. J., and Wright, G. W., *J. Clin. Invest.*, 33 530 (1954)
56. Frank, N. R., *J. Appl. Physiol.*, 14 905 (1959)
57. Fraser, M. S., *J. Obstet. Gynaecol. Brit. Empire*, 66, 748 (1959)
58. Gitlin, D., and Craig, J. M., *Pediatrics*, 17, 64 (1956)
59. Golinko, R. J., Rudolph, A. M., Auld, P. A. M., and Nelson, N. M., *Soc. Pediat. Research*, Abstr. #145 (May, 1960)
60. Graham, B. D., and Wilson, J. L., *Am. J. Diseases Children*, 87, 287 (1954)
61. Gribetz, I., Frank, N. R., and Avery, M. E., *J. Clin. Invest.*, 38, 2168 (1959)
62. Gruenwald, P., *Am. J. Obstet. Gynecol.*, 53, 996 (1957)
63. Heiner, D. C., and Sears, J. W., *J. Diseases Children* (Abstract to be published)
64. Hermann, L., *Arch. ges. physiol.*, 20, 365 (1879–80)
65. Hill, J. P., *J. Physiol.*, 149, 346 (1959)
66. Himwich, H. E., Bernstein, A. O., Herrlich, H., Chesler, A., and Fazekas, J. F., *Am. J. Physiol.*, 135, 387 (1942)
67. Hon, E. H., and Hulme, G. W., *Yale J. Biol. and Med.*, 31, 57 (1958–59)
68. James, L. S., Welsbrot, I. M., Prince, C. E., Holaday, D. A., and Apgar, V., *J. Pediat.*, 52, 379 (1958)
69. James, L. S., *Pediatrics*, 24, 1069 (1959)
70. James, L. S., Moya, F., Burnard, E. D., and Apgar, V., *Lancet, I:* 737 (1959)
71. James, L. S., *Acta Paediat.*, 49, Suppl. 122, 17 (1960)
72. Jäykka, S., *Acta Paediat.* 46, Suppl. 112 (1957)
73. Karlberg, P., *Acta Paediat.*, 41, Suppl. 89 (1952)
74. Karlberg, P., Cook, C. D., O'Brien, D., Cherry, R. B., and Smith, C. A., *Acta Paediat.*, 43, 397 (1954)
75. Karlberg, P., and Lind, J., *Semaine hôp.*, 32, 207 (1956)
76. Karlberg, P. J., *Josiah Macy Jr., Foundation*, 77–128 (1958)
77. Karlberg, P. J., Koch, G., Wallgren, G., and Geubelle, F., *Acta Paediat.*, 48, Suppl. 118, 128 (1959)
78. Karlberg, P. J., *J. Pediat.*, 56 (585) (1960)
79. Karlberg, P. J., Cherry, R. B., Escard6, F., and Koch, G., *Acta Paediat.*, 49, 345 (1960)
80. Kendig, E. L., *Pediatrics*, 26, 97 (1960)
81. Klaus, M., Tooley, W. H., Weaver, K. H., and Clements, J. A., *J. Diseases Children* (Abstract. To be published)
82. Landing, B. H., *Pediatrics*, 19, 217 (1957)
83. Lieberman, J., and Kellogg, F., *New Engl. J. Med.*, 262, 999 (1960)
84. Lind, J., *Acta Paediat.*, 49, Suppl. 122, 39 (1960)
85. Lind, J., *Die Physiologische Entwicklung Des Kindes Vorlesungen Über Kunktionelle Pädologie*, 105–28 (Springer-Verlag, Berlin, Göttingen, Heidelberg, Germany, 105 pp., 1959)
86. Loosli, C. G., and Potter, E. L., *Am. Rev. Respiratory Diseases*, 80, 5 (1959)
87. Mahaffey, L. W., and Rossdale, P. D., *Lancet*, I, 1223 (1959)
88. Miller, H. C., and Behrle, F. C., *Pediatrics*, 12, 141 (1953)
89. Moore, R. E., *J. Physiol.*, 149, 500 (1959)
90. Nelson, N. M., *Normal and Abnormal Respiration in Children* (Ross Conf. on Pediat. Research, 37th Conf., Kansas City, Kan., April, 1960. Ross Labs., Columbus, O.)
91. Pattle, R. E., *Proc. Royal Soc. (London)*, [B], 148, 217 (1958)
92. Potter, E. L., *Pathology of the Fetus and the Newborn*, 236 (The Year Book Publishers, Inc., Chicago, Illinois, 1952)
93. Ranck, J. B., and Windle, W. F., *Experimental Neurol.*, 1, 130 (1959)
94. Reardon, H. S., Field, S., Vega, L., Carrington, E., Arey, J., and Baumann, M. L., *J. Diseases Children*, 94, 558 (1957)
95. Reardon, H. S., *Adaption to Extrauterine Life* (Oliver, T. K., Ed., Ross Conf. on Pediat. Research, 31st Conf., Vancouver, B. C., 1959. Ross Labs., Columbus, O.)
96. Reardon, H. S., Baumann, M. L., and

6. Alvizouri, M., *Arch. Pathol.*, 66, 422 (1958)
7. Apgar, V., and Holaday, D. A. (Unpublished data)
8. Auld, P. A. M., Nelson, N. M., Cherry, R. B., and Smith, C. A., *J. Diseases Children* (Abstr. to be published)
9. Avery, M. E., Frank, N. R., and Gribetz, I., *J. Clin. Invest*, 38, 456–62 (1959)
10. Avery, M. E, and Mead, J., *J. Diseases Children*, 97; 517–(1959)
11. Avery, M. E., and Cook, C. D. (To be published)
12. Barcroft, J., *Researches on Pre-Natal Life* (Charles C Thomas, Publ., Springfield, Ill., 305 pp., 1958)
13. Bauman, W. A., *Pediatrics*, 24, 194 (1959)
14. Benirschke, K., *J. Diseases Children*, 99, 714 (1960)
15. Benson, F., and Celander, O., *Acta Paediat.*, 48, Suppl. 118, 49 (1959)
16. Berfenstam, R., Edlund, T., and Zettergren, L., *Acta Paediat.*, 47, 82 (1958)
17. Berglund, G., and Karlberg, P., *Acta Paediat.*, 45, 541 (1956)
18. Blanc, W. A., *Gynaecologia*, 136, 101 (1953)
19. Blystad, W., Landing, B. H., and Smith, C. A., *Pediatrics*, 8, 5 (1951)
20. Bondurant, S. *Normal and Abnormal normal Respiration in Children* (Ross Conf. on Pediat. Research, 37th Conf., Kansas City, Kan., April, 1960, Ross Labs, Columbus, O.)
21. Bosma, J. F., Fawcitt, J., Lind, J., Takagi, Y., and Wegelius, C., *Acta Paediat.*, 49, Suppl. 123 (1960)
22. Brandt, I. K., Cunningham, P., and Harned, H. S., Jr., *Pediatrics*, 25, 859 (1960)
23. Briggs, J. N., and Hogg, G., *Pediatrics*, 22, 41 (1958)
24. Brück, K., *Die Physiologische Entwicklung Des Kindes Vorlesunger Über Funktionelle Pädologie*, 41–53 (Springer-Verlag, Berlin, Göttingen, Heidelberg, Germany, 1959)
25. Bucci, G., *Minerva pediat.*, 12, 97 (1960)
26. Bundeson, H. N., *J. Am. Med. Assoc.*, 157, 1384 (1955)
27. Burnard, E. D., *Brit. Med. J.*, I, 134 (1959)
28. Burnard, E. D., *Brit. Med. J.*, I, 1495 (1959)
29. Calkins, L. A., and Miller, H. C., *Am. J. Obstet. Gynecol.*, 78, 1005 (1959)
30. Cohen, M. M., Weintraub, D. H., and Lilienfeld, A. M., *Pediatrics*, 26, 42 (1960)
31. Cook, C. D., Cherry, R. B. O'Brien, D., Karlberg, P., and Smith, C. A., *J. Clin. Invest.*, 34, 975 (1955)
32. Cook, C. D., Sutherland, J. M., Segal, S., Cherry, R. B., Mead, J., McIlroy, M. B., and Smith, C. A., *J. Clin. Invest.*, 36, 440 (1957)
33. Cook, C. D., Helliesen, P. J., and Agathon, S., *J. Appl. Physiol.*, 13, 349 (1958)
34. Cook, C. D., Barrie, H., and Avery, M. E., in *Advances in Pediat.*, 11, 50 (1960)
35. Cook, C. D., Bougas, T., Croteau, J. R., and Smith, R. M. (To be published)
36. Cook, C. D. (Unpublished data)
37. Cooper, E. A., Smith, H., and Pask, E. A., *Anaesthesia*, 15, 211 (1960)
38. Craig, J. M., Fenton, K., and Gitlin, D., *Pediatrics*, 22, 847 (1958)
39. Cross, K. W., *J. Physiol.*, 109, 459 (1949)
40. Cross, K. W., and Roberts, P. W., *Brit. Med. J.*, I, 1043, 1–13 (1951)
41. Cross, K. W., Tizard, J. P. M., and Trythall, D. A. H., *Acta Paediat.*, 46, 265 (1957)
42. Cross, K. W., Tizard, J. P. M.,and Trythall, D. A. H., *Acta Paediat.*, 47, 217 (1958)
43. Dawes, G. S., Mott, J. C., Widdicombe, J. G., and Wyatt, D. G., *J. Physiol.*, 12, 141 (1953)
44. Dawes, G. S., *Recent Advances in Paediatrics* (Gairdner, D., Ed., J. & A. Churchill, Ltd., London, Engl., 1958)
45. Dawes, G. S. *Arch. Diseases Childhood*, 34, 281 (1959)
46. Dawes, G. S., Mott, J. C., and Shelley, H. J., *J. Physiol.*, 146, 516 (1959)
47. Dawes, G. S., Mott, J. C., and Stafford, A., *J. Physiol.*, 153, 16 P (1960)
48. Drorbaugh, J. E., Segal, S., Sutherland, J. M., Cherry, R. B., Oppe, T. E., and Smith, C. A., *J. Diseases Children*, 90, 627 (1955)
49. Drorbaugh, J. E., "Physiology of Prematurity" 129, *Trans. Conf. Physiol. Prematurity, 2nd Conf.*, 1957 (The Josiah Macy, Jr., Foundation, New York, 1958)
50. Dubois, A. B., Botelho, S. Y., Bedell, G. N., Marshall, R., and Comroe,

EVALUATION OF PULMONARY FUNCTION: METHODS[1,2]

By Edward A. Gaensler, M.D.

The Thoracic Services, Massachusetts Memorial and Boston City Hospitals; Departments of Medicine and Surgery, Boston University School of Medicine, Boston, Massachusetts

Introduction

This review is intended to acquaint the reader with certain recent developments in methodology and with some new observations made possible by these technical advances. An attempt has been made to cover selectively the five-year period since Christie & Bates last reviewed this subject (1). Review of such a relatively long period precludes quotation of many excellent articles but may have the advantage of some added perspective. Publications primarily concerned with pulmonary circulation have been excluded; however, reference is made to a recent review in these pages (2) and elsewhere (3), and to an excellent symposium summarizing present knowledge (4). Studies of infants and children will be reviewed elsewhere in this volume.

Books and reviews.—The first books to appear since the war devoted wholly to pulmonary physiology have all been published recently. Foremost on this list is *The Lung* by Comroe and co-workers (5) which is, and for many years will be, the best text on the subject. Its brilliantly clear presentation and superb diagrams have set new standards for medical teaching. The text is not annotated and the section on clinical application is sketchy. A review of the English literature of the last fifteen years has been published by Knowles (6). Rossier and co-workers (7) have presented a fine text meticulously documented with data from their own long and extensive experience. Their hesitation to accept as gospel the fashions of the times is refreshing. Difficulties with a nomenclature of their own devising will be obviated by a forthcoming translation. *Clinical Cardiopulmonary Physiology* (8) is by far the most ambitious undertaking in this field. The second edition, to be published shortly, is a considerable improvement and has fine sections on "Evaluation of Pulmonary Physiology" and "Relation of Environmental Influences to Cardiopulmonary Physiology." The clinical sections, particularly on obstructive disease, are less worthwhile. Campbell's volume on the respiratory muscles deals with a much neglected subject in the needed mechanistic rather than the usual descriptive terms (9).

[1] The survey of the literature pertaining to this review covers the period January, 1955, to September, 1960.

[2] The following abbreviations will be used: A (alveolar gas phase); a (arterial blood phase); C.P.U.E. (capacité pulmonaire utilizable à l'effort); D_L (diffusing capacity of the lung); D_M (diffusing capacity of the membrane); FRC (functional residual capacity); MBC (maximum breathing capacity); MMF (maximal midexpiratory flow); P (partial pressure); V.C. (vital capacity); V_c (pulmonary capillary blood volume)

Haddad, E. J., *J. Pediat.*, 57, 151 (1960)

97. Rooth, G., and Sjöstedt, S., *Études Neo-natales*, 7, 121 (1958)
98. Rudolph, A. M., Auld, P. A. M., Drorbaugh, J. E., Rudolph, A. J., Nadas, A. S., and Smith, C. A., *Pediatrics* (Abstract. To be published)
99. Samartzis, E. A., Cook, C. D., and Rudolph, A. J., *Acta Paediat.* (To be published)
100. Schachter, F. F., and Apgar, V., *Pediatrics*, 24, 1016 (1959)
101. Shanklin, D. R., *Arch. Pathol.*, 68, 49 (1959)
102. Sherry, S., Lindemeyer, R. I., Fletcher, A. P., and Alkjaersig, N., *J. Clin. Invest.*, 38, 810 (1958)
103. Sjöstedt, S., and Rooth, G., *Arch. Diseases Childhood*, 32, 397 (1957)
104. Smith, C. A., and Chisholm, T., *Am. J. Diseases Children*, 62, 889 (1941)
105. Smith, C. A., *Ann. Paediat. Fenniae*, 4, 129, 147 (1958)
106. Snyder, F. F., *Am. J. Obstet Gynecol.*, 14, 730 (1959)
107. Sorokin, S., Padykula, H. A., and Herman, E., *Developmental Biol.*, 1, 125 (1959)
108. Stahlman, M. T., and Meece, N. J., *J. Clin. Invest.*, 36, 1081 (1957)
109. Stahlman, M. T., *J. Diseases Chidren* (Abstract. To be published)
110. Stahlman, M. T., *Normal and Abnormal Respiration in Children* (Ross Conf. on Pediat. Research, 37th Conf., Kansas City, Kan., 1960. Ross Labs., Columbus, O.)
111. Strang, L. B., Anderson, G. S., and Platt, J. W., *Lancet*, I, 954 (1957)
112. Swyer, P. R., Reiman, R. C., and Wright, J. J., *J. Pediat.*, 56, 612 (1960)
113. Usher, R., *Pediatrics*, 24, 562 (1959)
114. Usher, R., *J. Diseases Children* (Abstr. To be published, 1960)
115. Villee, C. A., Hagerman, D. D., Holmberg, N., Lind, J., and Villee, D. B., *Pediatrics*, 22, 953 (1958)
116. Villee, C. A., *Acta Paediat.*, 49, Suppl. 122, 5 (1960)
117. Weisbrot, I. M., James, L. S., Prince, C. E., Holaday, D. A., and Apgar, V., *J. Pediat.*, 52, 395 (1958)
118. Weisser, K., Cross, K. W., de Muth, G., Klaus, M., and Tooley, W. H. (To be published)
119. Westin, B., Nyberg, R., and Enhorning, G., *Acta Paediat.*, 47, 339 (1958)
120. Wilson, J. L., and Farber, S., *Am. J. Diseases Children*, 46, 590 (1933)
121. Wilson, M. G., and Mikity, V. G., *J Diseases Children*, 99, 489 (1960)
122. Ylppö, A., *Handbuch der Kinderheilkunde* I, 549 (Pfaundler-Schlossman, Leipsig, Germany, 1923)

and sex groups ranged between 3.4 and 11.8 l./sec. (29), and similar results were obtained for the volume exhaled from 280 to 1280 ml. (30). Another method for eliminating the first and last phases of the V. C. is the maximal midexpiratory flow (MMF) proposed by Leuallen & Fowler (31), which has found considerable acceptance. It is the average flow rate during expiration of the middle 50 per cent of the V. C. and the time required for this maneuver is the midexpiratory time. Like other timed segments, it is readily calculated from a recorded fast V. C. provided a suitable low-resistance spirometer with a fast kymograph speed is used. A simple V. C. machine with a small kymograph records the "Expirogram" (32).

Peak flow rate.—Renewed efforts are being made to measure the maximal flow rate. The "Puffmeter" of Goldsmith is, in essense, a pneumotachograph with the usual screen replaced by a cup-shaped, porous grinding wheel and the pressure transducer by a draft gauge (33, 34). Recorded "peak flows" averaging 293 l./min. for females and 368 l./min. for males are much lower than those recorded by conventional pneumotachography probably because it actually measures an average flow for a considerable time. The "peak" value is indicated for only an instant and humidity increases the resistance of the prime mover.

Other small devices for measuring maximal expiratory flow rate are the "pneumometer" of Hildebrant & Hanke (35) and the increasingly popular "peak flow meter" of Wright & McKerrow (36). The latter works much like a rotameter on the principle of a variable area orifice meter. A ratchet holds the pointer in position of maximum deflection which is the highest mean flow maintained for about 10 msec. Values are quite comparable to those obtained by pneumotachography, that is, about 700 l./min. in young males (37).

The maximum breathing capacity (MBC).—This test continues to be of considerable interest as evidenced by the many maneuvers proposed for its "indirect" measure (20 to 24). Unlike the single breath maneuvers, it appears to measure the integrity of the respiratory bellows as a whole and is affected by such factors as respiratory muscle blood supply, fatigue, progressive trapping, etc. Because of this, the MBC, in its relation to ventilatory requirement—the dyspnea index—correlates more closely with subjective dyspnea than does any other test (38, 39). Also because of this there are always individual exceptions to the good correlation between MBC and the various volume/time segments of the forced V. C. (19, 22). Attempts to relate the percentage of the timed vital capacity to the MBC volume (40) indicate a misconception of these tests. Consideration of the MBC, together with the maximum expiratory force (41) or esophageal pressure (42), allows for some definition of expiratory resistance.

Shephard (43) has made an extensive study of factors affecting the MBC. His conclusions that results are greatly affected by instrumental resistance, by variations in breathing rate, and by the learning effect, are not borne out by other studies. Cotes (44), Bartlett & Specht (45), and Zwi and co-workers (46) found no marked effect on the MBC with added expiratory resistance.

The massive volume of Bartels and co-workers (10) reviews comprehensively modern spirometry, ergometry, and all manner of blood and gas analyses supported by many valuable tables, compilations, and diagrams. The exhaustive bibliography in the best German tradition does much to reorient ideas concerning priority. This volume, together with *Instrumental Methods of Analysis* (11), and the book on electrical instrumentation by Lion (12) should be of interest to all concerned with instrumentation. The *Handbook of Respiration* (13), an extensive compilation of data, fills an important need and represents a very large amount of work by many. It tends to emphasize our lack of knowledge of certain normal values in man.

The "Medical Progress" articles in the *New England Journal of Medicine* (14, 15) and in *Progress in Cardiovascular Diseases* (16), and the commentary on the American Medical Association Pulmonary Function Exhibit (17) are shorter recent reviews.

Ventilatory Function Tests

The fast vital capacity: I. Timed volumes.—The shape and slope of the spirographically or otherwise recorded fast vital capacity (V.C.) has received increasing attention following the introduction of the timed vital capacity ten years ago (18, 19). The shorter time intervals are the more significant for estimation of ventilatory capacity. The first one-second interval was chosen independently in France and in this country (18, 19) but the ideal time is still under discussion: 0.5 (20), 0.75 (21), and even 2 sec. (22) have been recommended. Use of these time segments to calculate the "indirect maximal breathing capacity" stem from the repeatedly demonstrated close correlation between them and the maximal breathing capacity (18, 19, 21, 22). Tiffeneau (18) multiplied the one-second volume by 30 to obtain his C.P.U.E. while, at present, in France the preferred factor is 37.5 (23) and, in England, 40 (21). This reviewer fails to understand the justification for multiplying the results of one test by an arbitrary number and calling the new number by a different name, particularly one that implies performance of an entirely different test which is affected by other variables.

The conventional water spirometer for automatic electric recording of V. C. and timed volumes (19) has been modified for a bellows spirometer (24) with a stylus for recording of the expiratory slope (25, 26), and has been equipped with a calibrating device (27).

The fast vital capacity: II. Selected segments.—The earliest portion of the recorded fast V. C. is encumbered by patient hesitation and spirometer inertia; also, it may be misleadingly large because "trapping" does not occur instantly with the onset of forced expiration. Similarly, the flat, terminal, high-resistance phase is of little clinical importance. These considerations have led to elimination of the earliest and last portions from consideration. Cander & Comroe (28) calculated the mean expiratory flow for that portion of the V. C. exhaled between 200 and 1200 ml. Mean values for various age

and sex groups ranged between 3.4 and 11.8 l./sec. (29), and similar results were obtained for the volume exhaled from 280 to 1280 ml. (30). Another method for eliminating the first and last phases of the V. C. is the maximal midexpiratory flow (MMF) proposed by Leuallen & Fowler (31), which has found considerable acceptance. It is the average flow rate during expiration of the middle 50 per cent of the V. C. and the time required for this maneuver is the midexpiratory time. Like other timed segments, it is readily calculated from a recorded fast V. C. provided a suitable low-resistance spirometer with a fast kymograph speed is used. A simple V. C. machine with a small kymograph records the "Expirogram" (32).

Peak flow rate.—Renewed efforts are being made to measure the maximal flow rate. The "Puffmeter" of Goldsmith is, in essense, a pneumotachograph with the usual screen replaced by a cup-shaped, porous grinding wheel and the pressure transducer by a draft gauge (33, 34). Recorded "peak flows" averaging 293 l./min. for females and 368 l./min. for males are much lower than those recorded by conventional pneumotachography probably because it actually measures an average flow for a considerable time. The "peak" value is indicated for only an instant and humidity increases the resistance of the prime mover.

Other small devices for measuring maximal expiratory flow rate are the "pneumometer" of Hildebrant & Hanke (35) and the increasingly popular "peak flow meter" of Wright & McKerrow (36). The latter works much like a rotameter on the principle of a variable area orifice meter. A ratchet holds the pointer in position of maximum deflection which is the highest mean flow maintained for about 10 msec. Values are quite comparable to those obtained by pneumotachography, that is, about 700 l./min. in young males (37).

The maximum breathing capacity (MBC).—This test continues to be of considerable interest as evidenced by the many maneuvers proposed for its "indirect" measure (20 to 24). Unlike the single breath maneuvers, it appears to measure the integrity of the respiratory bellows as a whole and is affected by such factors as respiratory muscle blood supply, fatigue, progressive trapping, etc. Because of this, the MBC, in its relation to ventilatory requirement—the dyspnea index—correlates more closely with subjective dyspnea than does any other test (38, 39). Also because of this there are always individual exceptions to the good correlation between MBC and the various volume/time segments of the forced V. C. (19, 22). Attempts to relate the percentage of the timed vital capacity to the MBC volume (40) indicate a misconception of these tests. Consideration of the MBC, together with the maximum expirntory force (41) or esophageal pressure (42), allows for some definition of expiratory resistance.

Shephard (43) has made an extensive study of factors affecting the MBC. His conclusions that results are greatly affected by instrumental resistance, by variations in breathing rate, and by the learning effect, are not borne out by other studies. Cotes (44), Bartlett & Specht (45), and Zwi and co-workers (46) found no marked effect on the MBC with added expiratory resistance.

The type of high-velocity valve used, and whether open or closed circuit is employed, also appears to affect the MBC very little (46, 47). Choice of respiratory frequency within a wide range of 60 to 120 cycles per minute also does not significantly affect the outcome of the MBC as shown again by Ogilvie and his colleagues (42) and Milic-Emili & Petit (48) who, incidentally, also calculated the work of breathing during MBC. The spontaneously chosen respiratory rate leads to the largest MBC volume; and severest exercise ventilation, in their experieoce, never exceeded the MBC. The "learning effect," which we found to cause increments of 8 per cent between first and second tries in 1000 patients (49), was recently recorded as 7.5 per cent by Friend (50) who also found no significant variation in further tests made on the same day or in subsequent weeks and months.

The small "Ventube" of Warring & Siemsen (51) is a calibrated Venturi tube which, used in conjunction with a small rubber balloon, syringe, and calibration chart, can be used for MBC determinations with results comparing well with other techniques. A new valve for hyperventilation, designed by McKerrow & Otis (52), has one-half the resistance of the conventional Rudolph valve. Wright & Gilford (53) have designed an ingenious Krogh spirometer which permits direct read-out of MBC, timed V. C., various lung volumes, and distribution factors.

The old controversy between Krogh and Knipping coocerning the ideal design of spirometers, renewed some time ago in England by Mendel and D'Silva, has now been taken up in this country by Wells and his co-workers (54). Their new spirometer has a light plastic bell and is used without pulley or counterweight.

For the first time, quantitative evaluation of the "match test" has been attempted. This maneuver works quite well if the patient holds the mouth wide open while blowing (55) and if the distance between mouth and match is controlled (56).

Residual volume.—The functional residual capacity (FRC) is being measured more commonly by helium rebreathing, particularly since the easier helium-in-air method gives the same results as the old helium-in-oxygen technique (57). Oxygen addition only at the end of the procedure sacrifices the distribution index but simplifies the test (17, 58). Some find that the helium results deviate no more than 5 per cent from the nitrogen washout technique (59). Birath & Swenson have published a nomographic solution for the FRC calculation (60) and have measured the small quantity of helium taken up by the blood (61).

The gas pressure method for determination of the FRC has been revived. The older altitude chamber and explosive decompression have been replaced by a body plethysmograph, a small rigid chamber of constant volume (62). As the subject within the box breathes against an obstruction the gas is expanded or compressed within the thorax and pressure changes are plotted against airway pressure; from this relationship the volume of gas in the thorax can be calculated. In normal subjects, results are quite comparable to

those obtained by the nitrogen washout technique (63). As expected, in patients with trapped gas in the thorax (pneumothorax, cysts, or emphysema) the plethysmographic value is larger than the washout volume because the latter measures only communicating airspaces (64). This method, and other uses of the body plethysmograph, such as measurement of pulmonary capillary blood flow, airway resistance, and mechanical properties of the lungs and thorax, have been reviewed by DuBois (65). A constant-pressure, variable-volume plethysmograph has been described by Mead (66).

Nomenclature and normal values.—The "Pappenheimer Nomenclature" for lung volume compartments and respiratory symbols has found international acceptance (67). A new terminology suggested by the British Thoracic Society (68) for the many derivatives of the forced V. C. may help to clarify the methods used in published material but its complexity is not likely to help clinicians.

Normal values for ventilatory tests have been quoted up to date in the *Handbook* (13). A nomogram for prediction of V. C. for ages 17 to 80 has been constructed by Miller and co-workers (20) V. C. and timed V. C. in men over 40 has been recorded (69), and the close relationship between lung volumes and the third power of height has been reaffirmed (70). Lung volumes at an altitude of 5760 ft. were the same as at sea level, and MBC as well as ventilatory requirements were higher, and oxygen saturation lower (71). Results were comparable to Anderson's recorded at Denver, and those of Cotes (44). As everyone knows, prediction of MBC based on body surface area is not applicable in obese subjects (72).

Mechanics of Breathing

The large number of studies dealing with this subject published during the last five years includes a few short teaching reviews (15, 73) and excellent introductory chapters in books (5, 8, 10). An extensive review by Mead will appear shortly (74). Only a few recent developments can be touched upon here.

Simultaneous determinations of volume, rate of flow, and esophageal pressure have led to recent observations that have bearing on interpretation of some published results: (*a*) the vagaries of pleural and esophageal pressure measurement, (*b*) the mechanical factors in the distribution of pulmonary ventilation which may result in frequency dependence of compliance, and (*c*) the relationship of airway resistance to lung volume.

Intrapleural and intraesophageal pressure.—Buytendijk's demonstration that intraesophageal pressure can be used as a measure of intrapleural pressure has been exploited extensively most often in man and in the upright position. Comparison of the two pressures, however, has been made mostly in animals and in the supine position. Recent observations suggest caution in interpretation of both local intrapleural and intraesophageal pressures. First, earlier findings of Wiggers and his co-workers have been confirmed: the pleural pressure itself may differ 3 to 8 cm. in lateral and medial spaces

(75) and may be quite different at the apex and at the base (76). Secondly, esophageal and pleural pressures in man correspond closely in the upright position, but in the supine the esophageal pressure may differ from +60 to −25 per cent, and has an average greater amplitude of +27 per cent (77, 78), apparently because of the added weight of the mediastinal contents upon the esophagus. These inconsistencies vary with lung volume because of the action of the diaphragm upon the heart. With unilateral pleuropulmonary disease effects of positional changes are more marked on pleural than on esophageal pressures (79). Flow-resistive pressures correspond more closely than do elastic components of pressure; and in the supine position the compliance calculated from esophageal pressures may be falsely low (78). Studies of postural shifts in esophageal pressure and of pressure-volume curves in different positions have led to the same conclusions (80, 81). The problems of intraesophageal pressure measurements are illustrated by the often quoted finding of Bondurant and co-workers (82) that induced pulmonary congestion causes a decrease in compliance of 50 per cent. Esophageal pressure artefacts were largely responsible for this observation because the "G suit" used for production of central congestion distended the heart and great veins, thus causing high esophageal end-expiratory pressures much as in the supine position (83). Further, the suit shifted the pulmonary midposition in the expiratory direction by as much as a liter, thus reducing compliance by closure of air spaces (84, 85, 86).

Long (10 to 15 cm.) balloons are superior to short ones and air-filled systems lead to fewer artefacts than do those filled with water (87, 88). Methods for making balloons have been described (87, 89) and vinyl material has been recommended (90).

Mechanical factors in the distribution of pulmonary ventilation.—In normal subjects, the measured compliance remains essentially independent of frequency of breathing providing the level of the pulmonary midposition is carefully controlled. Mead and co-workers (91) observed that in emphysema compliance is larger than normal during airflow interruption, normal during quiet breathing, and as low as one-fifth of normal during rapid breathing. These findings, since confirmed (92, 93), suggest that patients with obstructive disease breathe with "different" lungs at different rates of breathing. These observations, experiments with lung models, and human bronchospirometric studies (94) prompted Otis and his colleagues (95) to make a theoretical analysis of the effects of local differences in mechanical properties on the distribution of ventilation within the lungs and on the lungs' overall mechanical behavior. Distribution of ventilation can be uninfluenced by changes in breathing frequency only if the time constants of separate pathways are the same. In a population of pathways, the mechanical properties of which are dissimilar but invariant with frequency, the behavior of the overall system must vary with the frequency of breathing even though the fundamental properties of each component remain unchanged. Experimentally, in normal adults, compliance can be made to drop with increasing frequency by

histamine; and, conversely, in asthmatics the frequency dependence of compliance may disappear by the administration of adrenalin (95).

Lung volume and airway resistance.—Related studies are extensions of observations of VonNeergard & Wirz, of Dayman, of Mead & Whittenberger, and of Fry and co-workers that airway resistance can be decreased by breathing with a voluntarily raised FRC., and becomes much greater than normal during lowering of the FRC. These alterations are said to be attributed to lateral forces, or "lung tension," acting upon the tracheobronchial tree. Campbell, Martin & Riley (96) remarked on the fact that expiratory forces acting on the alveolus must also bear externally on the relatively compressible air passages. During expiration the intraluminal pressure distal to the alveoli may therefore be less than the extraluminal pressure, thus tending to increase airway resistance. Thus, there is an intrathoracic pressure at a given lung volume which, if exceeded, does not result in a further increment of flow rates, the "maximum effective pressure"; the corresponding "maximum flow rate" is very high in normal subjects and severely reduced in obstructive disease. A variable resistance expiratory valve, mimicking these pressure-flow relationships, was constructed by Jones *et al.* to study the effect of various resuscitators (97).

Hyatt, Schilder & Fry (98) give further details of the functional relationship between transpulmonary pressure, respiratory gas flow, and degree of lung inflation in a series of sophisticated theoretical, mechanical, and experimental analyses (99). They find the relationship between maximal expiratory flow and degree of inflation primarily determined by the physical properties of the lower airways during the latter one-half of the V. C. (98, 100). Cheng and his co-workers, using a different technical approach, arrived at similar conclusions (101).

Air flow resistance.—This can be measured only if the rate of flow and the pressure drop from alveoli to mouth are known. For the problematical measure of alveolar pressure, Von Neergard & Wirz first suggested pressure recording at the mouth during brief periods of airway interruption. This pressure was thought to represent the pressure difference that existed between mouth and alveoli just prior to interruption. This assumption, further extended by Vuilleumier and by Otis & Proctor, was first questioned by Mead & Whittenberger (102) who found that interrupter airway resistance resulted in the same, or slightly larger, values than total (airway plus tissue) resistance measured by the pleural pressure method. They concluded that either there is no pressure equilibration during interruption, presumably because of continued activity of respiratory muscles and chest wall inertia, or tissue resistance must be non-existent. We now know that tissue resistance is not negligible (103, 104) yet the value of the interruption method is still not clarified. Clements *et al.* (105) improved upon an earlier interruption technique (106) for field testing of pulmonary resistance. Exhalation is made through a calibrated resistance with brief interruptions of air flow ten times a second. Airway resistance is calculated from the known resistance and the

airway pressure at momeots of interruption. Some have obtained quite irregular results with this valve (15) while others have appareotly used it successfully for study of the relationship between pulmooary resistance aod the state of lung inflation (101, 107).

Other recently developed methods for measuring airway resistance such as tbe body plethysmograph (65, 66, 104, 108), and indirect calculation from expiratory pressure and ventilatory data (31, 41, 42) have been mentioned.

Tissue viscous resistance.—The total pulmonary resistance is composed of the resistance offered by the airways to gas flow plus the resistance caused by the frictional loss involved in moving lung tissue; both are measured togetber by recording of (esophageal) pressure aod flow rate. Two recent methods have separated these two components. McIlroy and colleagues (103) measured tbe total resistaoce duriog breathing of gas mixtures of different physical properties which altered the airway resistance but oot the tissue resistance. They calculated that during quiet breathing, 30 to 40 per ceot of the total luog resistance results from tissue viscous resistance. Alternatively, the tissue viscous resistance can be calculated by subtracting from the total luog resistance determined by conventional methods the airway resistance alone measured by a different technique, for which Marshall & DuBois (104) chose the body plethysmograph. By this method it appeared that, in normal subjects, tissue viscous resistance constitutes only 10 to 30 per ceot of the total pulmonary resistaoce. In some pulmonary diseases, a moderate increase in tissue resistance was found (109).

Compliance of the thorax.—This can be calculated by subtracting luog compliance from the compliaoce of lungs and tborax obtained together. The latter has beeo estimated in the living only by pressure measurements at the mouth during voluntary relaxation against a closed airway. The suspicion that complete respiratory muscular relaxation cannot be achieved even by traioed subjects appears to have been confirmed by Nims and co-workers (110) who found in anesthetized, paralyzed subjects a lower tboracic compliance than in the cooscious state. Howell & Peckett (111) thought this was attributable to anesthesia because they found a decrease of compliance of 30 per cent whicb could not be confirmed by Foster, Heaf & Semple (112). At any rate, the findings of Rahn aod co-workers that compliance of the lungs and of thorax are approximately equal has now been confirmed (112, 113).

Inertance.—Rohrer calculated, oo the basis of anatomic data, that inertial forces are unimportant in tbe work of breathing. Recent work by Mead (114) aod by DuBois *et al.* (115, 116) confirmed that this "inertance" is indeed very small wheo compared to resistance and elastance. Measurements at increased ambient pressures further suggested that most of the inertance is io the air stream (114).

Surface tension.—Hysteresis is the behavior of a mechanical system in which the results of an applied force lags the force itself. Since the volume changes of the lung lag the transpulmonary pressure changes which produce them, the lungs may be said to manifest hysteresis. Surface tension phenom-

ena appear to be responsible for much of the lungs' hysteresis (117). Preliminary studies of material derived from the lung suggest that a low surface tension is an important attribute of the lining of the air passages (118, 119, 120). It has been suggested that alveolar structures are maintained in a balance between collapse and overdistention not only by tissue elements, but also by uniquely variable surface-active materials lining the alveolar walls. It may even be that certain pulmonary diseases may be caused initially by an excess (emphysema) or a deficiency (respiratory distress of the newborn) of this material, with visible cellular manifestations occurring only later (121). For example, lung extracts of small premature infants and those dying of hyaline membrane disease appeared to lack surface-active material (122).

From this it seems clear why, during several recent conferences on respiratory physiology, much time was devoted to the influence of surface tension on the stability of the air spaces (121, 123). Elementary considerations have been briefly reviewed by Mead (124), and Clements and his colleagues (125) have attempted to explain the dependence of the static pressure-volume characteristics on internal surface forces. Radford (126) concluded his excellent review on this subject: "The 'elastic' properties of the lungs depend largely on the true elastic elements and on surface forces. The interplay between surface and elastic properties of the lungs gives rise to highly complicated pressure-volume curves which show marked time-independent hysteresis, the magnitude of which depends on experimental conditions. Because of the complex anatomical arrangement of the elastic elements of the lung and the importance of surface tension, characterization of lung statics remains largely in the descriptive stage."

Normal values.—Reports of normal values for pulmonary compliance and resistance (127 to 131) are difficult to compare because of variable body position, methodology, and apparatus response (132), and the imprecision of all methods. Interpretation of findings in pulmonary disease relative to these normal standards is not always clear because of the added variables of altered lung volume and breathing frequencies.

EXCHANGE OF GASES BETWEEN ALVEOLAR AIR AND PULMONARY BLOOD

Diffusing capacity for oxygen.—The diffusing capacity of the lungs is defined as the amount of gas which crosses the membrane per unit of time, divided by the pressure difference of that gas across the membrane. In the case of oxygen the diffused quantity of gas is readily ascertained and a measure of the mean alveolar oxygen tension can be obtained at least in normal subjects. For estimating the mean capillary oxygen tension we must know not only the alveolar oxygen tension, the shape of the oxygen dissociation curve and the mixed venous oxygen tension but also the end-capillary oxygen tension. Since there is no way of sampling end-capillary blood it was not until Lilienthal & Riley developed their brilliant method for indirect calculation of this pressure that diffusing capacity for oxygen could be estimated. Much fascinating and imaginative work has been accomplished with this

technique, but it is difficult, presents many theoretical, clinical, and analytical problems, and is not applicable unless certain experimental conditions are rigorously adhered to. The most critical measurement, the alveolar-to-end-capillary oxygen tension difference, has in normal subjects at rest an error as great as the value itself. This limits the usefulness of this technique to exercise conditions or to patients with abnormally large alveolar-arterial (A-a) oxygen tension differences. Furthermore, the necessary low oxygen breathing introduces uncertainties because it is not known whether venous admixture and diffusing capacity remain unchanged, and cardiac output and oxygen uptake often do change. Recently, the oxygen method has been of interest chiefly in relation to the miximal diffusing capacity, limitations upon exercise imposed by the diffusing capacity and comparisons with the carbon monoxide techniques (133 to 136).

Several of the theoretical problems, many of the technical difficulties, and the tedious trial and error calculations of the O_2 technique would be obviated if measurements of the A-a O_2 difference could be made at an alveolar O_2 tension high enough that the membrane component would become negligible. Present bubble equilibration techniques are not suitable for measurement of oxygen tensions much above 100 mm. Hg, but newly developed electrochemical methods may be and have been used in this connection (137 to 141). Advances in oxygen polarography may permit measurement of oxygen tensions in whole blood during oxygen breathing and may greatly facilitate an approach which was first pursued so laboriously by Berggren 20 years ago.

Diffusing capacity for carbon monoxide.—The vast amount of recent work concerned with diffusing capacity has been stimulated largely by revival of the carbon monoxide (CO) techniques prompted by publications of Forster *et al.* (142), Filley and his colleagues (143), and perhaps Kruhøffer (144). The use of CO offers an advantage in that its affinity for hemoglobin is so great that end-capillary CO tension has been considered negligible. Development of a simple, precise, and specific method for CO analysis by non-dispersion infrared spectroscopy has permitted experimentation on a scale unthinkable for Bohr or the Kroghs, and has provided a new clinical tool for functional evaluation of lungs. Tests and measurements have become technically simple, yet, as more experience is gained, interpretation of results has become more difficult and the meaning of "diffusing capacity" is now being closely re-examined.

Fine introductions to this subject are now available (5, 10, 145) and Forster (146) has written an excellent review which is pleasant reading even for the uninitiated.

Two of the most interesting aspects of diffusing capacity which until now have not been evaluated quantitatively are the separate contributions to total diffusion resistance offered by the "membrane" itself and by intracapillary resistances, and the effects of uneven ventilation and perfusion on the measured diffusing capacity.

Exchange across the membrane and diffusion and chemical combination within the blood.—Roughton & Forster and their colleagues have opened a

new era by showing that it is possible to separate resistance to diffusion of gases across the lung into a membrane and a blood component (147 to 150). Basing their studies, in part, upon earlier work of Roughton they demonstrated that the rate at which the hemoglobin in the red cell takes up CO to form carboxyhemoglobin (COHb) is so slow that this reaction speed may limit gas exchange. Carbon monoxide diffuses across the membrane into plasma where its tension increases until it can diffuse into the red cell at the same rate as it enters the plasma. They find that, when breathing 21 per cent O_2, the tension of CO in the plasma is about one-half of its pressure in the alveolar gas. Thus, the resistance to gas movement across the membrane is about equal to the "intracapillary" resistance to the entrance of gas into red cells. The diffusing capacity of the lung, D_L, has then two components: the diffusing capacity of the membrane, D_M, and the rate at which the pulmonary capillary blood can take up CO. The latter depends upon the pulmonary capillary blood volume, V_C, and the velocity θ, with which blood can form COHb. V_C and D_M can be calculated by measurement of $D_{L_{CO}}$ at different alveolar oxygen tensions (150).

A similar analysis for the diffusion of O_2 has been presented by Mochizuki & Fukuoka (151) in which they conclude that membrance resistance is only one-fourth that of red cell resistance; they are supported in this by Lewis *et al.* (152).

The mechanisms whereby disease may produce a predominant change in the intracapillary resistance to diffusion have been discussed by Forster (146). In normal subjects, McNeill and his co-workers (153) found average values of 97 ml. for V_C and 64 ml./min./mm. Hg for D_M. The important contributions of chemical reaction rate or pulmonary capillary blood volume, or both on D_L are now under investigation. Anemia causes a reduction of D_L while polycythemia increases it (151, 154). Patients with cardiac septal defects without pulmonary hypertension have an abnormally large D_L (155, 156), which appears to be caused entirely by an increase of V_C (123, 153). In pulmonary fibrosis, D_M is much more reduced than is V_C (153, 157). The finding of normal V_C in severe emphysema (153) obviously will require confirmation.

Effects of non-uniformity of lung on estimation of diffusing capacity.—Increasing technical precision of measurements of diffusing capacity and other physiologic variables no longer permits ignoring the fact that the D_L expression may be incorrect in the presence of lung non-uniformity (38, 142). The four primary variables that may affect estimates of D_L are alveolar volume, alveolar ventilation, pulmonary blood flow, and D_L. Which of the six possible ratios of these variables in individual alveoli may affect D_L has been tabulated by Forster (146). In emphysema, for example, the ventilation/perfusion ratios may be as high as 2.24 in well-ventilated alveoli and as low as 0.23 in poorly ventilated alveoli, with corresponding end-capillary oxygen saturations of 97.5 and 76 per cent. From such data, Briscoe *et al.* (158) conclude that inhomogeneity of this severity invalidates D_L calculations based upon any single mean alveolar O_2 or CO tension, and alveolar ventilation based on equating alveolar P_{CO_2} with arterial P_{CO_2}. Because of this some in-

vestigators have not performed measurements of D_L in patients who showed any evidence of airway obstruction or measurable unevenness of pulmonary ventilation (159, 160). Yet, others have studied emphysema patients by various D_L measurements (161, 162, 163) and have even drawn deductions concerning pathogenesis (164) or attached prognostic significance to the outcome of the tests (165). It is becoming increasingly apparent that end-tidal sampling techniques measure the combined effect of poor diffusion and unequal ventilation (145, 146, 166), and that all methods of D_L measurement, in the presence of markedly non-uniform lung, are not suitable for measuring the condition of the pulmonary capillary bed, its area, its thickness, or its hemoglobin content, that is, the potential to exchange gas. Perhaps these measurements do give an indication of whether or not the useful diffusion properties of the lung are altered. I believe that in this latter connection the term "diffusing capacity" is misleading.

Comparison of different methods for evaluation of diffusing capacity.—Three CO techniques have found wide acceptance: (*a*) the modification of the single-breath technique proposed by Forster and co-workers (142) in which initial alveolar P_{CO} is calculated from helium dilution; (*b*) the physiologic dead-space, steady-state modification of Filley and his colleagues (143) in which alveolar P_{CO} is calculated from the Bohr equation assuming equality for arterial and alveolar P_{CO_2}; and (*c*) the end-tidal sampling steady-state modification of Bates and his co-workers (167) in which alveolar P_{CO} is measured directly.

A number of comparisons of these methods have now been made in normal subjects and in patients with pulmonary disease (143, 160, 161, 166 to 170). The values for the CO steady-state techniques agree fairly closely with those obtained by the O_2 method when correction is made for the theoretical relation of $D_{M_{O_2}} = 1.23 \times D_{M_{CO}}$ as suggested by Krogh. Whether this relationship actually holds for $D_{L_{O_2}}$ and $D_{L_{CO}}$ has been questioned in view of the possibly differing rates of combination of CO and O_2 with intracellular hemoglobin (146, 147, 148, 161).

The single-breath value is nearly twice as large as that obtained by the steady-state methods; the single dissenting finding (169) appears to have been caused by laboratory error (171). M. Krogh first found that the single-breath D_L decreases as the volume at which the breath is held is reduced. Although Forster and his collaborators have not been able to confirm this (146, 168, 172), others have found a consistent diminution of D_L with lung volume (160, 166, 170, 173). Hence, in normal subjects the difference between steady-state and single-breath D_L may be caused by differences in lung volume at which the tests are made, but differences in "back pressure" and capillary blood volume cannot be excluded (174). The difference between the two tests is greater in patients with impaired diffusing capacity (160) and greatest in emphysema (145, 166). A nearly linear relationship between the ratio, single-breath D_L/steady-state D_L, and the degree of unevenness of intrapulmonary distribution of gas can be demonstrated (174).

Simplifications of the various methods have been suggested, among them portable, box-bag apparatus for field testing of D_L (175), and modifications of Kruhøffer's rebreathing method (144). The factors affecting the rate of CO uptake have been of interest for many years. Recently, a number of clinicians have been fairly satisfied with the diagnostic and prognostic value of CO uptake itself in that, under certain conditions, this value correlates very well with measures of diffusing capacity; corrections of this "fraction of CO removed" have been suggested for physiologic dead-space or minute ventilation (143, 160, 161, 163, 169, 176, 177).

The effect upon diffusing capacity of exercise, hyperventilation, increased pulmonary blood flow, and increased pulmonary capillary blood volume.—The long established finding that D_L increases during exercise has been amply confirmed in normal subjects and, with one exception (165), in patients with pulmonary disease (133 to 136, 140, 142, 143, 156, 159, 160, 173, 178, 179). The factors responsible for this increase are not clear. However, under physiologic circumstances their interplay is so complicated as to make distinction very difficult: ventilation, pulmonary blood flow, and possibly pulmonary capillary blood volume, all increase during exercise. Interestingly, during voluntary hyperventilation D_L increases as much or more than during exercise that elicits comparable ventilation (156, 180, 181). On the other hand, if blood flow is increased (by drugs) or decreased (by unilateral occlusion of one pulmonary artery) there appears to be no effect on the diffusing capacity (180, 181). Furthermore, in excised lungs D_L is not affected by changes of blood flow as long as the mean intravascular pressure remains constant (182).

When intravascular pressure is allowed to rise by raising the left atrial pressure while keeping blood flow constant, or by increasing blood flow while keeping left atrial outflow level, D_L does increase. From this Rosenberg & Forster (182) concluded that the pressure across the walls of the blood vessels is a primary factor in controlling the size of the capillary bed as measured by D_L. Other experiments leading to a change of pulmonary capillary blood volume appear to support this conclusion. Although Lewis and his collaborators (183) found no change in D_L upon inflation of a G suit, probably because of altered lung volume and closure of certain air spaces (83, 83, 86), Ross and co-workers (184) found a significant increase in D_L when central venous pressure was increased under controlled conditions. Valsalva maneuvers tend to decrease D_L while Müller maneuvers tend to increase it (172, 184). Indeed, the Müller-like maneuver immediately preceding the breath-holding test may be responsible, in part, for the larger value of D_L obtained by this method (174). Negative intra-alveolar pressure causes a marked increase of D_L and V_0 but not of D_M (185). Johnson *et al.* (179) demonstrated that during exercise D_L, D_M, and V_C are closely related to pulmonary capillary blood flow, suggesting that both volume and effective surface of the pulmonary capillary bed change with corresponding directional changes in blood flow.

Normal values.—Values for the single-breath and the alveolar sampling

methods have been published (172, 186, 187); there are few normal data for the physiologic dead-space method because of the required arterial blood (143, 159, 160). The prediction formula for "maximal oxygen D_L" (136) appears to be applicable to severe exercise steady-state CO. Diffusing capacity of the lung is related to body surface area, sex, and age (136, 167, 172, 186, 187, 188).

Analytical Methods

Physical methods of gas and blood analysis are rapidly replacing chemical methods. The principles upon which these analyses are based are not new. The recent effective utilization of these principles has been made possible largely by improved sensing devices such as photomultipliers, thermistors, and condenser-membranes, and by advances in electronics such as scanning circuits and highly stable power supplies and amplifiers.

Many times physical analyzers are installed in the spirit of automation with the hope of saving personnel, time, and money. Often these hopes are not realized because the equipment is expensive to purchase, install, and maintain and because elaborate calibration by biochemical methods is often required. However, a brief discussion of the newest methods appears justified because their capabilities particularly in regard to speed of response, continuous analysis, and freedom from interference have made possible new observations that have increased our understanding of normal and abnormal respiratory physiology. Established examples are the infrared and Hopcalite CO meters which have stimulated great advances in our understanding of diffusing capacity; or the glow discharge nitrogen meter which has altered our thinking concerning intrapulmonary distribution of gas, and has made possible simple determination of the anatomic dead-space. Mass spectrometry, infrared spectroscopy and partition chromatography for gas analysis, polarography for whole blood analysis, and the use of radioactive gas isotopes will be reviewed, together with some observations that would not have been possible without them.

Mass spectrometry.—Faulconer, in his introduction to blood gas analysis, said that "with some 'poetic license' the mass spectrometer might be described as a physical instrument capable of sorting and counting molecules in a sample from complex mixtures of gases" (189). The molecules of the sample are ionized at low pressure by a beam of electrons and the ions are deflected in a circular path by a magnetic field; the stream of particles splits into beams of different molecular weight, any one of which can be detected by a suitably placed collector; a moving collector produces a "mass spectrum." First mention of this method for respiratory gas analysis was made by Hitchcock & Stacy (190) who showed that during expiration alveolar gas concentrations change continuously. Technical details of mass spectrometers have been described by Siri (191) and by Nier (192) who designed the machine described by Faulconer (189), and who worked with Miller and others (193) on respiratory CO_2 analysis during anesthesia. A commercial mass spectrometer at the University of Pennsylvania has been used by Comroe, Bartels, DuBois,

Forster and others for monitoring of respiratory and a variety of tracer gases (142, 150, 157, 172, 194, etc.).

Theoretically, the mass spectrometer is capable of analyzing any gas, regardless of the composition of diluting gases, with a speed, specificity, sensitivity, and accuracy unmatched by any other known method of analysis. However, the large size, the need for water cooling of the diffusion pump, liquid nitrogen cooling of the vapor-trap, and other problems of continuous high-vacuum operation, and the great expense of such instruments has precluded their more widespread use; even when available, the continued supervision and maintenance problems have been disheartening. Two attempts in this country to produce commercial, small, portable, relatively inexpensive mass spectrometers have failed largely because the required short-cuts deprived the instruments of their stability and quantitative analytical capabilities.

Recently, Fowler & Hugh-Jones (195) described a mass spectrometer specifically designed for respiratory work. Like previous devices, it requires only 15 ml./min. for analysis, has a response time of 0.1 sec. for 95 per cent deflection with an overall accuracy of 3 per cent. In addition, it can analyze simultaneously and continuously four gases by electronic scanning of the available spectrum 25 times per second; it presents analyses on an oscilloscope, by meter deflection, and on a direct writing recorder; and water and liquid air cooling is dispensed with. West *et al.* have made extensive use of this instrument for instantaneous measurements of respiratory exchange ratio and ventilation-perfusion inequalities (196) and for endobronchial gas tension and flow analysis (197). From experimental observations in dogs (198) they were able to study in man characteristic patterns of local changes in O_2, CO_2, and introduced argon from which they recognized partial bronchial obstruction or arterial obstruction, or both, in different lobes and segments (199). Endobronchial flow rate and pendulum movement of air was measured from intensity of deflection of an argon stream introduced across a fine gap of an instrument within the bronchus (200).

Infrared gas analysis.—Non-dispersion, infrared spectroscopy can be used for detection of gases with polyatomic molecules including CO, CO_2, H_2O vapor, N_2O, and organic anesthetic gases. Description of the principle has been ascribed to Luft (201) and has been reviewed by Carlson (202). This method has found wide acceptance because instruments are unusually stable, reliable, trouble-free and relatively small and inexpensive.

Great sensitivity can be achieved with large analysis chambers and greater than atmospheric sampling pressure. The carbon monoxide meter referred to previously is sensitive to 1 p.p.m. and has been used for nearly all recent work on D_{LCO} (142, 150, 154 to 157, 159 to 177, etc.). In addition, new methods have been developed for carboxyhemoglobin analysis for which the blood is decomposed, all gas extracted by vacuum, and its CO concentration measured with the meter (203, 204); the lungs can also be used as a tonometer with calculation of COHb from equilibrated alveolar gas (205, 206, 207).

If sensitivity is not required, the analysis chamber can be made extremely

small and thus a very rapid response can be achieved which enables continuous analysis of expired carbon dioxide with a speed that approaches that of the mass spectrometer. A variety of methods for alveolar P_{CO_2} analysis were described in detail by Collier and co-workers (208) and critically evaluated by Cara (209). The experimental and clinical importance of this application can hardly be overstated.

In anesthesia, the CO_2 meter has made it possible to monitor the adequacy of ventilation without need for educated guessing, and allowed circumvention of the considerable technical difficulties of direct measurement of alveolar ventilation (210, 211).

In obstructive pulmonary disease, end-tidal sampling is of limited value because deductions of arterial P_{CO_2} from "alveolar" P_{CO_2} are not possible. This difficulty can be circumvented by rebreathing of four to six breaths of about 8 per cent CO_2 during which time the gas in the bag, in the lungs, and in the mixed venous blood come into equilibrium (212). The CO_2 meter monitors the equilibration procedure and analyzes the final mixed sample. Subtraction of 6 mm. Hg from the mixed venous P_{CO_2} gives an acceptable estimate of arterial P_{CO_2} even in patients with severe heart disease or emphysema. Variations of this method have been used clinically to circumvent the more difficult analyses for arterial P_{CO_2} (214, 215).

Infrared CO_2 meters are playing an exclusive role in the recent measurement of the arterial-alveolar (a-A) CO_2 pressure difference. Ordinarily, for calculation of alveolar P_{O_2}, or P_{CO_2}, and in other applications of the alveolar equation, arterial P_{CO_2} is equated to alveolar P_{CO_2} (5, 7, 143). The error of this assumption in patients with severely uneven ventilation and perfusion has been forcefully re-emphasized by Briscoe and co-workers (158). However, even in normal subjects and experimental animals, there is a small a-A P_{CO_2} gradient (208, 216, 217) which is probably attributable entirely to normal ventilation-perfusion discrepancies and not to a diffusion gradient. Nevertheless, because of the Haldane effect, it is probably incorrect to say that CO_2 diffuses 25 times more rapidly than O_2 (218). The mean alveolar P_{CO_2} is not easily determined because it rises continuously as expiration progresses (190, 208, 209). This and analytical difficulties have prevented exact definition of the normal a-A P_{CO_2} difference (218).

Uneven distribution of pulmonary blood flow results in an alveolar dead-space and also in an abnormal a-A P_{CO_2} difference. Severinghaus & Stupfel (216) demonstrated this by creating non-uniformity of perfusion by pulmonary air emboli; Leigh *et al.* (219) showed a similar effect with local diminution of pulmonary blood flow during great vessel surgery; and Robin and his collaborators (220, 221, 222), in much publicized studies, showed the development of a considerable a-A P_{CO_2} gradient in pulmonary embolism and suggested measurement of the gradient for clinical diagnosis and quantitative evaluation of this catastrophe.

Gas chromatography.—According to Keulemans' monograph (223), "Chromatography is a physical method of separation, in which the compo-

nents to be separated are distributed between two phases, one constituting a stationary bed of large surface area, the other being a fluid that percolates along." Martin & Synge (224) proposed that a gas could be used as the mobile phase, and later demonstrated gas-liquid partition chromatography (225). Application of gas chromatography to analysis of respiratory gases is new (226, 227) and is the only method in this section with which this reviewer has had no personal experience.

Helium, at a controlled flow rate, is commonly used as carrier gas. One or more packing columns separate the components of the test gas and qualitative identification is made by appearance time. Each component is then analyzed in sequence in a thermistor thermal conductivity cell.

Commercial instruments, with minor modifications, have good precision (228) and, with zero suppression, may equal the accuracy of the Scholander analyzer (229). Each analysis requires from several minutes to one-half hour. However, a large variety of gases including the chemically inert can be measured (229) and blood gas analysis may also be possible. The method has been used for determination of ventilation-perfusion ratios (230) and of diffusing capacity (231).

Polarographic determination of P_{O_2}.—Oxygen saturation as an index of blood oxygenation is often quite unsatisfactory both from the technical and physiologic standpoints. Procedures on the Van Slyke machine are long and tedious and spectrophotometric or reflectometric methods are relatively inaccurate. More important, because of the shape of the O_2 dissociation curve, no conclusions concerning P_{O_2} can be drawn from saturations above 80 per cent. The interest in direct oxygen tension measurements is therefore not surprising and tonometric methods date back to 1870. However, even recent "bubble" modifications of tonometry have remained difficult and time-consuming. Polarographic techniques which permit speedy, direct P_{O_2} measurement in small samples of whole blood at any O_2 tension are likely to broaden our knowledge of respiratory and exercise physiology and may revolutionize clinical laboratory examination.

Polarography is a careful electrolysis of solutions containing reducible or oxidizable substances. When a suitable voltage is applied to an electrode immersed in a solution containing O_2, the O_2 is reduced (i.e., takes up electrons). This transfer of electrons can be measured by a galvanometer and the current flowing is a function of the P_{O_2}. A platinum electrode works well enough in tissues but in whole blood "electrode poisoning" occurs; for most previous work, cumbersome anaerobic centrifugation was required for polarography of cell-free plasma. Dropping mercury electrodes have a surface which is continually renewed, thus circumventing poisoning. Unfortunately, even the newest modifications by Bartels (232, 233) require calibration curves with samples of known oxygen tension for each individual blood. Although this last method has been used extensively (139, 140, 141), it is time consuming and has been completely unsatisfactory in our hands.

Introduction of the hydrophobic membrane-covered (usually polyethyl-

ene) platinum-silver reduction cell by Clark (234), Drenckhahn (235), and Reeves and co-workers (236) was a great improvement because the platinum cathode was separated from contamination with other chemical constituents of the sample. Oxygen tension gradients within the liquid sample caused by diffusion of oxygen across the membrane is a problem. One solution is the use of very permeable membranes with a response so rapid that continuous recording of transient blood P_{O_2} is possible (237, 238). Another solution is the stirring of a relatively large sample (239 to 243). And a third approach has been the use of a relatively impermeable (Mylar) membrane (244) which reduces oxygen transfer so much that stirring is unnecessary and a very small blood sample (0.1 ml.) suffices; this is at the expense of a low output and prolonged response time.

The many modifications and the present preoccupation with design suggest that O_2 polarography has not yet come of age. However, the method holds great promise. A variety of clinical applications has been illustrated by Miller and co-workers (245), and others were mentioned in the section on oxygen diffusing capacity.

Electrometric measurements of P_{CO_2}.—For direct P_{CO_2} measurements, the Astrup method of determining pH once on the unknown and once on the same blood equilibrated at known P_{CO_2}, is now being replaced by the use of CO_2 electrodes described by Severinghaus & Bradley (240) and by Snell (246).

Radioactive gas isotopes.—Only the briefest mention can be made of recent work with gas isotopes. Despite enthusiastic reports, the use of these gases has serious limitations: metabolic gases with long half-life must be used with great caution, and, as with $C^{14}O$ (144), may offer no obvious advantages. Use of the 2-min. half-life O_2^{15}, CO^{15}, and CO_2^{15} is limited to institutions that have a pile or cyclotron on the campus (247), and a radiation hazard cannot be discounted particularly with repeated examinations. External counting presents very special problems and appears to be semiquantitative at best.

Knipping *et al.* (248) have reported on "Isotopenthorakographie" with xenon 133; and Dyson and collaborators (247) have assessed regional lung function with oxygen 15. The increasing external count upon inspiration of O_2^{15} is taken as a measure of ventilation and the decay during breath-holding as a measure of perfusion. Simultaneous comparison of the two lungs, or upper and lower lung fields, is made more accurate by annihilation coincidence counting (249). The claims of Knipping (248) and of Dyson (247) and their colleagues that this procedure is safer and simpler than bronchospirometry cannot be taken too seriously. Similar work with CO_2^{15} has led West & Dollery (250) to conclude that the variation in blood flow between upper and lower parts of the lung in erect man accounts for the whole of the ventilation-perfusion inequality in the normal lung. That the diffusing capacity for O_2^{15} is greater than for O_2^{16} appears surprising at first but is easily explained by Dyson and co-workers (251).

Krypton 85 has been used for some years as inspired gas for the measure-

ment of organ blood flow, study of exchange of inert gases, and for detection of intracardiac shunts. Despite its long half-life, its use is safe because it is inert and has a very low solubility in blood (252). Recently, it has been administered intravenously and only about 5 per cent appeared in the systemic arteries (253). The problem of recirculation was so minimal that right ventricular output could be measured continuously. Application of krypton 85 to the study of the ventilation-perfusion relationship promises to be most interesting (254, 255).

LITERATURE CITED

1. Christie, R. V., and Bates, D. V., *Ann. Rev. Med.*, 6, 211 (1955)
2. Liebow, A. A., *Ann. Rev. Med.*, 11, 95 (1960)
3. Donnet, V., and Ardisson, J. L., *J. physiol. (Paris)*, 50, 587 (1958)
4. Adams, W. R., and Veith, I., Eds., *Pulmonary Circulation* (Grune & Stratton, Inc., New York, 316 pp., 1959)
5. Comroe, J. H., Jr., Forster, R. E., DuBois, A. B., Briscoe, W. A., and Carlson, E., *The Lung: Clinical Physiology and Pulmonary Function Tests* (The Year Book Publishers, Inc., Chicago, Ill., 219 pp., 1955)
6. Knowles, J. H., *Respiratory Physiology and Its Clinical Application* (Harvard University Press, Cambridge., Mass., 256 pp., 1959)
7. Rossier, P. H., Bühlman, A., and Wiesinger, K., *Physiologie und Pathophysiologie der Atmung*, 2nd ed. (Springer-Verlag, Berlin, Germany, 330 pp., 1958); Translated by Luchsinger, P. C., and Moser, K. M., *Respiration* (The C. V. Mosby Co., St. Louis, Mo., 505 pp., 1960)
8. Gordon, B. L., Ed., *Clinical Cardiopulmonary Physiology* (Grune & Stratton, Inc., New York, 759 pp., 1957)
9. Campbell, E. J. M., *The Respiratory Muscles and the Mechanics of Breathing* (The Year Book Publishers, Inc., Chicago, Ill., 131 pp., 1958)
10. Bartels, H., Bücherl, E., Hertz, C. W., Rodewald, G., and Schwab, M., *Lungenfunktionsprüfungen* (Springer-Verlag, Berlin, Germany, 426 pp., 1959)
11. Willard, H. H., Merritt, L. L., and Dean, J. A., *Instrumental Methods of Analysis*, 3rd. ed. (D. Van Nostrand Co., Inc., Princeton, 626 pp., 1958)
12. Lion, K. S., *Instrumentation in Scientific Research: Electrical Input Transducers* (McGraw-Hill Book Co., Inc., New York, 462 pp., 1959)
13. Dittmer, D. S., and Grebe, R. M. Eds., *Handbook of Respiration* (W. B. Saunders Co., Philadelphia, Pa., 403 pp., 1958)
14. Gaensler, E. A., *New Engl. J. Med.*, 252, 177, 221, 264 (1955)
15. Ferris, B. G., Jr., *New Engl. J. Med.*, 262, 557, 610 (1960)
16. Friedberg, C. K., Ed., *Progr. in Cardiovascular Diseases*, 1, 251, 341 (1959)
17. Meneely, G. R., *Diseases of Chest*, 31, 125 (1957)
18. Tiffeneau, R., Bousser, J., and Drutel, R., *Paris méd.*, 137, 543 (1949)
19. Gaensler, E. A., *Am. Rev. Tuberc.*, 64, 256 (1951)
20. Miller, W. F., Johnson, R. L., and Wu, N., *Diseases of Chest*, 30, 33 (1956)
21. Kennedy, M. C. S., *Thorax*, 8, 73 (1953)
22. Needham, C. D., Rogan, M. C., and McDonald, I., *Brit. J. Tuberc., Rept. No. 450*, 1 (1955)
23. Cara, M., *Poumon Coeur*, 9, 406 (1953)
24. Shephard, R. J., Thomson, M. L., Carey, G. C. R., and Phair, J. J., *J. Appl. Physiol.*, 13, 189 (1958)
25. Wang, R. I. H., and Shipley, R. E., *J. Am. Med. Assoc.*, 167, 1730 (1958)
26. Horton, G. E., and Phillips, S., *Am. Rev. Respiratory Diseases*, 80, 724 (1959)
27. McKerrow, C. B., McDermott, M., and Gilson, J. C., *Lancet*, I, 149 (1960)
28. Cander, L., and Comroe, J. H., Jr., *J. Allergy*, 26, 210 (1955)
29. Arkins, J. A., Glaser, M. R., and Tret-

tel, R. J., *Diseases of Chest*, 37, 496 (1960)
30. March, H. W., and Lyons, H. A., *Diseases of Chest*, 37, 602 (1960)
31. Leuallen, E. C., and Fowler, W. S., *Am. Rev. Tuberc. Pulmonary Diseases*, 72, 783 (1955)
32. Franklin, W., Michelson, A. L., Lowell, F. C., and Schiller, I. W., *New Engl. J. Med.*, 253, 799 (1955)
33. Goldsmith, J. R., and Young, A. C., *J. Appl. Physiol.*, 8, 562 (1956)
34. Goldsmith, J. R., *Am. Rev. Tuberc. Pulmonary Diseases*, 78, 180 (1958)
35. Hildebrant, G., and Hanke, O., *Ärztl. Wochschr.*, 11, 439 (1956)
36. Wright, B. M., and McKerrow, C. B., *Brit. Med. J.*, II, 1041 (1959)
37. Higgins, I. T. T., *Brit. Med. J.*, II, 1198 (1957)
38. Gilson, J. C., and Hugh-Jones, P., *Brit. Med. Res. Council; Special Rept. Ser. #290* (Her Majesty's Stationery Office, London, England, 1955)
39. Fletcher, C., *Trans. Med. Soc. London*, 74, 55 (1958)
40. Anderson, W. H., *Diseases of Chest*, 38, 370 (1960)
41. McGregor, M., *Am. Rev. Tuberc. Pulmonary Diseases*, 78, 692 (1958)
42. Ogilvie, C. M., Stone, R. W., and Marshall, R., *Clin. Sci.*, 14, 101 (1955)
43. Shephard, R. J., *Flying Personnel Research Comm. Rept. #976* (Air Ministry, Inst. of Aviation Med., Royal Air Force, Farnborough, Engl., 1956)
44. Cotes, J. E., *Proc. Roy. Soc. (London)*, [B], 143, 32 (1954)
45. Bartlett, R. G., Jr., and Specht, H., *J. Appl. Physiol.*, 11, 79 (1957)
46. Zwi, S., Theron, J. C., McGregor, M., Becklake, M. R., *Diseases of Chest*, 36, 361 (1959)
47. Stuart, D. G., and Cohen, A. A., *Am. Rev. Tuberc. Pulmonary Diseases*, 79, 253 (1959)
48. Milic-Emili, G., and Petit, J. M., *Boll. soc. ital. biol. sper.*, 35, 431 (1959)
49. Gaensler, E. A., Rayl, D. F., and Donnelly, D. M., *Surg., Gynecol. Obstet.*, 92, 81 (1951)
50. Friend, J., *Thorax*, 10, 359 (1955)
51. Warring, F. C., Jr., and Siemsen, J. K., *Am. Rev. Tuberc. Pulmonary Diseases*, 75, 303 (1957)
52. McKerrow, C. B., and Otis, A. B., *J. Appl. Physiol.*, 9, 497 (1956)
53. Wright, G. W., and Gilford, S. R., *J. Thoracic Surg.*, 38, 643 (1959)
54. Wells, H. S., Stead, W. W., Rossing, T. D., and Ognanovich, J., *J. Appl. Physiol.*, 14, 451 (1959)
55. Snider, T. H., Stevens, J. P., Wilner, F. M., and Lewis, B. M., *J. Am. Med. Assoc.*, 170, 1631 (1959)
56. Jones, R. H., *New Engl. J. Med.* (To be published, 1961)
57. Weiner, R. S., and Cooper, P., *Am. Rev. Tuberc. Pulmonary Diseases*, 74, 729 (1956)
58. DiSalvo, R. J., and Goto, U., *Diseases of Chest*, 36, 624 (1959)
59. Motley, H. L., *Am. Rev. Tuberc. Pulmonary Diseases*, 76, 601 (1957)
60. Birath, G., and Swenson, E. W., *Scand. J. Clin. & Lab. Invest.*, 8, 329 (1956)
61. Birath, G. and Swenson, E. W., *Scand. J. Clin. & Lab Invest.*, 8, 155 (1956)
62. Comroe, J. H., Jr., Botelho, S. Y., and DuBois, A. B., *J. Appl. Physiol.*, 14, 439 (1959)
63. DuBois, A. B., Botelho, S. Y., Bedell, G. N., Marshall, R., and Comroe, J. H., Jr., *J. Clin. Invest.*, 35, 322 (1956)
64. Bedell, G. N., Marshall, R., DuBois, A. B., and Comroe, J. H., Jr., *J. Clin. Invest.*, 35, 664 (1956)
65. DuBois, A. B., *Physiologist*, 2, 8 (1959)
66. Mead, J., *J. Appl. Physiol.*, 15, 736 (1960)
67. "Abschliessender Bericht der Kommission der Deutschen Gesellschaft für Innere Medizin zur Normung der Nomenklatur und der Symbole von Atmungsgrössen," *Kongr. ges. inn. Med.*, 192, 1 (1957)
68. Gandevia, B., and Hugh-Jones, P., *Thorax*, 12, 290 (1957)
69. Pemberton, J., and Flanagan, E. G., *J. Appl. Physiol.*, 9, 219 (1956)
70. Hepper, N. G. G., Fowler, W. S., and Helmholz, H. F., Jr., *Diseases of Chest*, 37, 314 (1960)
71. Goldman, H. I., and Becklake, M. R., *Am. Rev. Tuberc. Pulmonary Diseases*, 79, 457 (1959)
72. Leiner, G. C., and Abramowitz, S., *Am. Rev. Respiratory Diseases*, 80, 902 (1959)
73. Gaensler, E. A., and Lindgren, I., *Progr. in Cardiovascular Diseases*, 1, 397 (1959)
74. Mead, J., *Physiol. Revs.* (To be published, 1961)
75. Coleridge, J. C. G., and Linden, R. J., *J. Physiol. (London)*, 126, 304 (1954)

76. Fahri, L. Otis, A. B., and Proctor, D. F., *School of Aviation Med., USAF, Publication #56-27* (San Antonio, Tex., 1956)
77. Butler, J., White, H. C., and Arnott, W. M., *Clin. Sci.*, 16, 709 (1957)
78. Mead, J., and Gaensler, E. A., *J. Appl. Physiol.*, 14, 81 (1959)
79. Attinger, E. O., Monroe, R. G., and Segal, M. S., *J. Clin. Invest.*, 35, 904 (1956)
80. Ferris, B. G., Jr., Mead, J., and Frank, N. R., *J. Appl. Physiol.*, 14, 521 (1959)
81. Knowles, J. H., Hong, S. K., and Rahn, H., *J. Appl. Physiol.*, 14, 525 (1959)
82. Bondurant, S., Hickam, J. B., and Isley, J. K., *J. Clin. Invest.*, 36, 59 (1957)
83. Bondurant, S., Mead, J., and Cook, C. D., *J. Appl. Physiol.*, 15, 875 (1960)
84. Bernstein, L., *J. Physiol. (London)*, 138, 473 (1957)
85. Mead, J., and Collier, C., *J. Appl. Physiol.*, 14, 669 (1959)
86. Ferris, B. G., Jr., and Pollard, D. S., *J. Clin. Invest.*, 39, 143 (1960)
87. Mead, J., McIlroy, M. B., Selverstone, N. J., and Kriete, B. C., *J. Appl. Physiol.*, 7, 491 (1955)
88. Petit, J. M., and Milic-Emili, G., *J. Appl. Physiol.*, 13, 481 (1958)
89. Schilder, D. P., Hyatt, R. E., and Fry, D. L., *J. Appl. Physiol.*, 14, 1057 (1959)
90. Crane, M. G., Affeldt, J. E., Austin, E., and Bower, A. G., *J. Appl. Physiol.*, 9, 11 (1956)
91. Mead, J., Lindgren, I., and Gaensler, E. A., *J. Clin. Invest.*, 34, 1005 (1955)
92. Attinger, E., Herschfus, J., and Segal, M. S., *J. Clin. Invest.*, 35, 912 (1956)
93. Saxton, G. A., Jr., Rabinowitz, M., Dexter, L., and Haynes, F., *J. Clin. Invest.*, 35, 611 (1956)
94. McKerrow, C. B., Otis, A. B., Bartlett, R. A., and Armstrong, B., *School of Aviation Med., USAF, Report #55-117* (San Antonio, Tex., 1956)
95. Otis, A. B., McKerrow, C. B., Bartlett, R., Mead, J., McIlroy, M. B., Selverstone, N. J., and Radford, E. P., *J. Appl. Physiol.*, 8, 427 (1956)
96. Campbell, E. J. M., Martin, H. B., and Riley, R. L., *Bull. Johns Hopkins Hosp.*, 101, 329 (1957)
97. Jones, R. H., Macnamara, J., and Gaensler, E. A., *Am. Rev. Respiratory Diseases*, 82, 164 (1960)
98. Hyatt, R. E., Schilder, D. P., and Fry, D. L., *J. Appl. Physiol.*, 13, 331 (1958)
99. Fry, D. L., *Phys. in Med. Biol.*, 3, 174 (1958)
100. Fry, D. L., and Hyatt, R. E., *Am. J. Med.*, 29, 672 (1960)-
101. Cheng, T. O., Godfrey, M. P., and Shepard, R. H., *J. Appl. Physiol.*, 14, 727 (1959)
102. Mead, J., and Whittenberger, J. L., *J. Appl. Physiol.*, 6, 408 (1954)
103. McIlroy, M. B., Mead, J., Selverstone, N. J., and Radford, E. P., Jr., *J. Appl. Physiol.*, 7, 485 (1955)
104. Marshall, R., and DuBois, A. B., *Clin. Sci.*, 15, 161 (1956)
105. Clements, J. A., Sharp, J. T., Johnson, R. P., and Elam, J. O., *J. Clin. Invest.*, 38, 1262 (1959)
106. Ainsworth, M., and Eveleigh, J. W., Ministry of Supply, C.D.E.E. *Porton Technical Paper, 320* (Her Majesty's Stationery Office, London, Engl., 1952)
107. Shephard, R., *J. Physiol. (London)*, 145, 459 (1959)
108. DuBois, A. B., Botelho, S. Y., and Comroe, J. H., Jr., *J. Clin. Invest.*, 35, 327 (1956)
109. Marshall, R., and DuBois, A. B., *Clin. Sci.*, 15, 473 (1956)
110. Nims, R. G., Conner, J. H., and Comroe, J. H., Jr., *J. Clin. Invest.*, 34, 774 (1955)
111. Howell, J. B. L., and Peckett, B. W., *J. Physiol. (London)*, 136, 1 (1957)
112. Foster, C. A., Heaf, P. J. D., and Semple, S. J. G., *J. Appl. Physiol.*, 11, 383 (1957)
113. Heaf, P. J. D., and Prime, F. J., *Clin. Sci.*, 15, 319 (1956)
114. Mead, J., *J. Appl. Physiol.*, 9, 208 (1956)
115. DuBois, A. B., Brady, A., Lewis, D., and Burgess, B., Jr., *J. Appl. Physiol.*, 8, 587 (1956)
116. Brady, A., DuBois, A., Nisell, O., and Engelberg, J., *Am. J. Physiol.*, 186, 142 (1956)
117. Mead, J., Whittenberger, J. L., and Radford, E. P., Jr., *J. Appl. Physiol.*, 10, 191 (1957)
118. Pattle, R. E., *Proc. Roy. Soc. (London)* [B], 148, 217 (1958)
119. Clements, J. A., *Proc. Soc. Exptl. Biol. Med.*, 95, 170 (1957)
120. Brown, E. S., *Proc. Soc. Exptl. Biol. Med.*, 95, 168 (1957)

121. Proc. Second Aspen Conf. on Research in Emphysema, *Am. Rev. Respiratory Diseases*, 81, 734 (1960)
122. Avery, M. E., and Mead, J., *J. Diseases Children*, 97, 517 (1959)
123. Ross Laboratories Conference in Pediatric Research, Kansas City, Kansas, 1960 (To be published, 1961)
124. Mead, J., *Am. Rev. Respiratory Diseases*, 81, 739 (1960)
125. Clements, J. A., Brown, E. S., and Johnson, R. P., *J. Appl. Physiol.*, 12, 262 (1958)
126. Radford, E. P., Jr., *Tissue Elasticity*, 177 (Am. Physiol. Soc., Washington, D.C., 1957)
127. Frank, N. R., Mead, J., Siebens, A. A., and Storey, C. F., *J. Appl. Physiol.*, 9, 38 (1956)
128. Butler, J., White, H. C., and Arnott, W. M., *Clin. Sci.*, 16, 709 (1957)
129. Frank, N. R., Mead, J., and Ferris, B. G., Jr., *J. Clin. Invest.*, 36, 1680 (1957)
130. Marshall, R., *Clin. Sci.*, 16, 507 (1957)
131. Briscoe, W. A., and DuBois, A. G., *J. Clin. Invest.*, 37, 1279 (1958)
132. Fry, D. L., Hyatt, R. E., McCall, C. B., and Mallos, A. J., *J. Appl. Physiol.*, 10, 210 (1957)
133. Shepard, R. H., *J. Appl. Physiol.*, 12, 487 (1958)
134. Shepard, R. H., Varnauskas, E., Martin, H. B., White, H. A., Permutt, S., Cotes, J. E., and Riley, R. L., *J. Appl. Physiol.*, 13, 205 (1958)
135. Linderholm, H., *Acta Med. Scand.*, 163, 61 (1959)
136. Cohn, J. E., Carroll, D. G., Armstrong, B. W., Shepard, R. H., and Riley, R. L., *J. Appl. Physiol.*, 6, 573 (1954)
137. Wiesinger, K., *Helv. Physiol. et Pharmacol. Acta*, Suppl. 7 (1950)
138. Wilson, R. H., Ebert, R. V., Borden, C. W., Pearson, R. T., Johnson, R. S., Falk, A., and Dempsey, M. E., *Am. Rev. Tuberc.*, 68, 177 (1953)
139. Bartels, H., Beer, R., Koepchen, H. P., Wenner, J., and Witt, I., *Arch. ges. Physiol.*, 261, 133 (1955)
140. Bartels, H., Koepchen, H. P., Lubning, I., Mochizuki, M., and Witt, I., *Arch. ges. Physiol.*, 261, 535 (1955)
141. Bartels, H., Beer, R., Fleischer, E., Hoffheinz, H. J., Krall, J., Rodewald, G., Wenner, J., and Witt, I., *Arch. ges. Physiol.*, 261, 99 (1955)
142. Forster, R. E., Fowler, W. S., Bates, D. V., and Van Lingen, B., *J. Clin. Invest.*, 33, 1135 (1954)
143. Filley, G. F., MacIntosh, D. J., and Wright, G. W., *J. Clin. Invest.*, 33, 530 (1954)
144. Kruhøffer, P., *Acta Physiol. Scand.*, 32, 106 (1954)
145. Forster, R. E., *Progr. in Cardiovascular Diseases*, 1, 268 (1959)
146. Forster, R. E., *Physiol. Revs.*, 37, 391 (1957)
147. Forster, R. E., Roughton, F. J. W., Kreuzer, F., and Briscoe, W. A., *J. Appl. Physiol.*, 11, 260 (1957)
148. Roughton, F. J. W., Forster, R. E., and Cander, L., *J. Appl. Physiol.*, 11, 269 (1957)
149. Forster, R. E., Roughton, F. J. W., Cander, L., Briscoe, A., and Kreuzer, F., *J. Appl. Physiol.*, 11, 277 (1957)
150. Roughton, F. J. W., and Forster, R. E., *J. Appl. Physiol*, 11, 290 (1957)
151. Mochizuki, M., and Fukuoka, J., *Japan. J. Physiol.*, 8, 206 (1958)
152. Lewis, B. M., Lin, T. H., Noe, F. E., and Komisaruk, R., *J. Clin. Invest*, 37, 1061 (1958)
153. McNeill, R. S., Rankin, J., and Forster, R. E., *Clin. Sci.*, 17, 465 (1958)
154. Kilburn, K. H., *Ann. Rev. Respiratory Diseases*, 81, 945 (1960)
155. Bedell, G. N., *Ann. Rev. Respiratory Diseases*, 81, 946 (1960)
156. MacNamara, J., Prime, F. J., and Sinclair, J. D., *Lancet*, I, 404 (1960)
157. Rankin, J., McNeill, R. S., and Forster, R. E., *J. Clin. Invest.*, 36, 922 (1957)
158. Briscoe, W. A., Cree, E. M., Filler, J., Houssay, H. E., Jr., and Cournand, A., *J. Appl. Physiol.*, 15, 785 (1960)
159. Cugell, D. W., Marks, A., Ellicott, M. F., Badger, T. L., and Gaensler, E. A., *Am. Rev. Tuberc. Pulmonary Diseases*, 74, 317 (1956)
160. Marks, A., Cugell, D. W., Cadigan, J. B., and Gaensler, E. A., *Am. J. Med.*, 22, 51 (1957)
161. Bates, D. V., *J. Clin. Invest.*, 37, 591 (1958)
162. Williams, M. H., Jr., and Zohman, L. R., *Am. Rev. Respiratory Diseases*, 80, 689 (1959)
163. MacNamara, J., Prime, F. J., and Sinclair, J. D., *Thorax*, 14, 166 (1959)
164. Ogilvie, C. M., *Thorax*, 14, 113 (1959)
165. Bates, D. V., Knott, J. M. S., and

Christie, R. V., *Quart. J. Med.*, 25, 137 (1956)
166. Marshall, R., *J. Clin. Invest.*, 37, 394 (1958)
167. Bates, D. V., Boucot, N. G., and Dormer, A. E., *J. Physiol. (London)* 129, 237 (1955)
168. Forster, R. E., Cohn, J. E., Briscoe, W. A., Blakemore, W. S., and Riley, R. L., *J. Clin. Invest.*, 34, 1417 (1955)
169. Bates, D. V., and Pearce, J. F., *J. Physiol. (London)*, 132, 232 (1956)
170. Shephard, R. J., *J. Physiol. (London)*, 141, 408 (1958)
171. Bates, D. V., (Personal communications to the author)
172. Ogilvie, C. M., Forster, R. E., Blakemore, W. S., and Morton, J. W., *J. Clin. Invest.*, 36, 1 (1957)
173. Gaensler, E. A., Verstraeten, J. M., Weil, W. B., Cugell, D. W., Marks, A., Cadigan, J. B., Jones, R. H., and Ellicott, M. F., *Arch. Ind. Health*, 19, 132 (1959)
174. Cadigan, J. B., Marks, A., Ellicott, M. F., Jones, R. H., and Gaensler, E. A., *J. Clin. Invest.* (To be published, 1961)
175. Shephard, R. J., Carey, G. C. R., and Phair, J. J., *J. Appl. Physiol.*, 12, 79 (1958)
176. Carey, G. C. R., Phair, J. J., Shepard, R. J., and Thomson, M. L., *Arch. Ind. Health*, 16, 225 (1957)
177. Williams, M. H., Jr., and Zohman, L., *Am. Rev. Tuberc. Pulmonary Diseases*, 78, 173 (1958)
178. Siebens, A. A., Frank, N. R., Kent, D. C., Newman, M. M., Rauf, R. A., and Vestal, B. L., *Am. Rev. Respiratory Diseases*, 80, 806 (1959)
179. Johnson, R. L., Jr., Spicer, W. S., Bishop, J. M., and Forster, R. E., *J. Appl. Physiol.*, 15, 893 (1960)
180. Ross, J. C., Frayser, R., and Hickam, J. B., *J. Clin. Invest.*, 38, 916 (1959)
181. Turino, G. M., Brandfonbrener, M., and Fishman, A. P., *J. Clin. Invest.*, 38, 1186 (1959)
182. Rosenberg, E., and Forster, R. E., *J. Appl. Physiol.*, 15, 883 (1960)
183. Lewis, B. M., Forster, R. E., and Beckman, E. L., *Clin. Research Proc.*, 4, 150 (1956)
184. Ross, J. C., Lord, T. H., and Levy, G. D., *J. Appl. Physiol.*, 15, 843 (1960)
185. Cotes, J. E., Snidal, D. P., and Shepard, R. H., *J. Appl. Physiol.*, 15, 372 (1960)
186. Stahlman, M. T., *J. Clin. Invest.*, 36, 1081 (1957)
187. Donevan, R. E., Palmer, W. H., Varvis, C. J., and Bates, D. V., *J. Appl. Physiol.*, 14, 483 (1959)
188. Bucci, G., and Cook, C. D., *Am. J. Diseases Children*, 100, 742 (1960)
189. Faulconer, A., *Anesthesiology*, 14, 405 (1953)
190. Hitchcock, F. A., and Stacy, R. W., *Am. J. Physiol.*, 155, 443 (1948)
191. Siri, W., *Rev. Sci. Instr.*, 18, 540 (1947)
192. Nier, A. O., *Rev. Sci. Instr.*, 11, 212 (1940); 18, 398 (1947)
193. Miller, F. A., Hemmingway, A., Nier, A. O., Knight, R. J., Brown, E. B., and Varco, R. L., *J. Thoracic Surg.*, 20, 714 (1950)
194. Bartels, J., Severinghaus, J. W., Forster, R. E., Briscoe, W. A., and Bates, D. V., *J. Clin. Invest.*, 33, 41 (1954)
195. Fowler, K. T., and Hugh-Jones, P., *Brit. Med. J.*, I, 1205 (1957)
196. West, J. B., Fowler, K. T., Hugh-Jones, P., and O'Donnell, T. V., *Clin. Sci.*, 16, 529 (1957)
197. West, J. B., and Hugh-Jones, P., *J. Appl. Physiol.*, 14, 753 (1959)
198. West, J. B., and Hugh-Jones, P., *J. Appl. Physiol.*, 14, 743 (1959)
199. Hugh-Jones, P., and West, J. B., *Thorax*, 15, 154 (1960)
200. West, J. B., *J. Appl. Physiol.*, 15, 976 (1960)
201. Luft, K. F., *Z. tech. Physik*, 24, 97 (1943)
202. Carlson, L. D., "Respiratory Exchange," in *Methods of Medical Research*, VI, 69–70 (The Year Book Publishers, Inc., Chicago, Ill., 1954)
203. Lawther, P. J., and Apthorp, G. H., *Brit. J. Ind. Med.*, 12, 326 (1955)
204. Gaensler, E. A., Cadigan, J. B., Ellicott, M. F., Jones, R. H., and Marks, A., *J. Lab. Clin. Med.*, 49, 945 (1957)
205. Carlsten, A., Holmgren, P., Linroth, K., Sjöstrand, T., and Ström, G., *Acta Physiol. Scand.*, 31, 62 (1954)
206. Linderholm, H., *Acta Med. Scand.*, 156, 413 (1957)
207. Jones, R. H., Ellicott, M. F., Cadigan, J. B., and Gaensler, E. A., *J. Lab. Clin. Med.*, 51, 553 (1958)
208. Collier, C. R., Affeldt, J. E., and Farr, A. F., *J. Lab. Clin. Med.*, 45, 526 (1956)
209. Cara, M., *Anesthésie et analgésie*, 14, 311 (1957)
210. Siebecker, K. L., Mendenhall, J. T.,

and Emanuel, D. A., *J. Thoracic Surg.*, 27, 468 (1954)
211. Elam, J. O., and Brown, E. S., *Anesthesiology*, 17, 116 (1956)
212. Collier, C. R., *J. Appl. Physiol.*, 9, 25 (1956)
213. Hackney, J. D., Sears, C. H., and Collier, C. R., *J. Appl. Physiol.*, 12, 425 (1958)
214. Griggs, D. E., Hackney, J. D., Collier, C. R., and Affeldt, J. E., *Am. J. Med.*, 25, 31 (1958)
215. Campbell, E. J. M., and Howell, J. B. L., *Brit. Med. J.*, I, 458 (1960)
216. Severinghaus, J. W., and Stupfel, M., *J. Appl. Physiol.*, 10, 335 (1957)
217. Lambertsen, C. J., and Benjamin, J. M., Jr., *J. Appl. Physiol.*, 14, 711 (1959)
218. Entretiens de Physio-Pathologie Respiratoire, IV[me] sér., Faculté de Médecin (Nancy, France, October 2–4, 1960)
219. Leigh, M. D., Jenkins, L. C., Belton, M. K., and Lewis, G. B , Jr., *Anesthesiology*, 18, 878 (1957)
220. Robin, E. D., Julian, D. G., Travis, D. M., and Crump, C. H., *New Engl. J. Med.*, 260, 586 (1959)
221. Robin, E. D., Forkner, C. E., Jr., Bromberg, P. A., Croteau, J. R., and Travis, D. M., *New Engl. J. Med.*, 262, 283 (1960)
222. Julian, D. G., Travis, D. M., Robin, E. D., and Crump, C. H., *J. Appl. Physiol.*, 15, 87 (1960)
223. Keulemans, A. I. M., *Gas Chromatography*, 2nd ed. (Reinhold Publ. Corp , New York, N. Y., 1959)
224. Martin, A. J. P., and Synge, R. L. M., *Biochem. J.*, 35, 1358 (1941)
225. James, A. T., and Martin, A. J. P., *Analyst*, 77, 915 (1952)
226. Brenner, N., and Cieplinski, E., *Ann. N. Y. Acad. Sci.*, 72, 13 , 705 (1959)
227. Hamilton, L. H., *Physiologist*, 2, 51 (1959)
228. Dressler, D. P., Mastio, G. J., and Albritten, F. F., Jr., *J. Lab. Clin. Med.*, 55, 144 (1960)
229. Hamilton, L. H., and Kory, R. C., *J. Appl. Physiol.*, 15, 829 (1960)
230 Lawson, W. H., Jr., and Johnson, R. L., Jr., *Am. Rev. Respiratory Diseases*, 81, 943 (1960)
231. Boren, H. G., and Kracke, F. L., *Am. Rev. Respiratory Diseases*, 81, 944 (1960)
232. Bartels, H., Burger, W., Eschweiler, W., and Laue, D., *Arch. ges. Physiol*, 254, 137 (1951)
233. Bartels, H., *Arch. ges. Physiol.*, 254, 107 (1951)
234. Clark, L. C., *Trans. Am. Soc. Artificial Internal Organs*, 2, 41 (1956)
235. Drenckhahn, F. O., *Arch. ges. Physiol.*, 262, 169 (1956)
236. Reeves, R. B., Rennie, D. W., and Pappenheimer, J. R., *Federation Proc.*, 16, 693 (1957)
237. Clark, L. C., Wolf, R., Granger, D., and Taylor, Z., *J. Appl. Physiol.*, 6, 189 (1953)
238. Tsao, M. U., and Vadnay, A., *J. Appl. Physiol.*, 15, 712 (1960)
239. Sproule, B. J., Miller, W. F., Cushing, I. E., and Chapman, S. B., *J. Appl. Physiol.*, 11, 365 (1957)
240. Severinghaus, J. W., and Bradley, A. F., *J. Appl. Physiol.*, 13, 515 (1958)
241. Kreuzer, F., Watson, T. R., and Ball, J. M., *J. Appl. Physiol*, 12, 65 (1958)
242. Rooth, G., Sjöstedt, S., and Caligara, F., *Clin. Sci.*, 18, 379 (1959)
243. Bartels, H., and Reinhardt, W., *Arch. ges. Physiol.*, 271, 105 (1960)
244. Polgar, G., and Forster, R. E., *J. Appl Physiol.*, 15, 706 (1960)
245. Miller, W. F., Sproule, B. J., and Cushing, I. E., *Am. Rev. Tuberc. Pulmonary Diseases*, 79, 315 (1959)
246. Snell, F. M., *J. Appl. Physiol.*, 15, 729 (1960)
247. Dyson, N. A., Hugh-Jones, P., Newbery, G. R., Sinclair, J. D., and West, J. B , *Brit. Med. J.*, I, 231 (1960)
248. Knipping, H. W., Bolt, W., Valentin, M., Venrath, H., and Endler, P., *Münch. med. Wochschr.*, 99, 1 (1957)
249. Dyson, N. A., *Phys. in Med. Biol.*, 4, 376 (1959–60)
250. West, J. B., and Dollery, C. T., *J. Appl. Physiol.*, 15, 405 (1960)
251. Dyson, N. A., Sinclair, J. D., and West, J. B., *J. Physiol. (London)*, 152, 325 (1960)
252. Hardewig, A., Rochester, D. F., and Briscoe, W. A., *J. Appl. Physiol.*, 15, 723 (1960)
253. Chidsey, C. A , III, Fritts, H. W., Jr., Hardewig, A., Richards, D. W., and Cournand, A., *J. Appl. Physiol.*, 14, 63 (1959)
254. Briscoe, W. A., Hardewig, A., Emmanuel, G., Gurtner, H. P., Rochester, D. F., and Cournand, A., *Federation Proc.*, 28, 59 (1959)
255. Gurtner, H. P., Briscoe, W. A., and Cournand, A., *J. Clin. Invest.*, 39, 1080 (1960)

AUTHOR INDEX

D

E

F

Y

SUBJECT INDEX

W

X

Z

CUMULATIVE INDEXES

VOLUMES 8 TO 12

INDEX OF CONTRIBUTING AUTHORS

INDEX OF CHAPTER TITLES